Analysis of Multipesticide Residues in Tobacco

The Agilent Triple Quadrupole GC/MS/MS Analyzer for Pesticides in Tobacco

Application Note

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Abstract

A multiple reaction monitoring (MRM) method was developed and evaluated on an Agilent 7000C Triple Quadrupole GC/MS for the analysis of 162 multiclass pesticides in tobacco. This system was equipped with a Multimode Inlet, and injections of 1 µL were made in the splitless mode. The midcolumn backflushing technique was applied for time-effective elimination of less volatile matrix components from the GC column. This reduced the cycle time and prevented contamination of the MS ion source. A modified QuEChERS sample preparation technique was used. Numerous tobacco samples were analyzed for method validation. Key performance parameters investigated were linearity, recovery, %RSD, limit of detection (LOD), and limit of quantitation (LOQ).

Assuming the water content in all tobacco samples was zero, this study showed excellent recoveries (70 to 120%) for 95% of all test pesticides at 0.05 and 0.5 mg/kg (pesticide:tobacco, w:w), and LOQs of 0.01 mg/kg or lower for most pesticides. We obtained excellent linearity from 0.01 to 2 mg/kg (pesticide:tobacco, w:w), and repeatability over seven injections at concentration levels near LOQ, and at 0.1 mg/kg in a tobacco matrix.

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Introduction

Tobacco, one of the world's leading high-value crops, is prone to pest attack, so farmers apply various pesticides as control measures. Pesticide residues on tobacco during cultivation can remain in the leaves at harvest and even survive postharvest processing treatments, eventually appearing in the final products. Concerns regarding threats to human health from the use of pesticides have prompted the tobacco industry worldwide to put more emphasis on the risks of pesticide residues in tobacco. To protect consumers and control pesticide residue levels, Guidance Residue Levels (GRLs) for 118 pesticides have been issued [1].

Multipesticide residue analysis in tobacco is a challenge in both sample preparation and analytical detection. Tandem mass spectrometry (MS/MS) on a triple quadrupole platform is very useful for screening, confirming, and quantitating trace-level target compounds in these complex matrices because it can minimize interferences. GC/MS/MS techniques provide much better sensitivity and, thus, significantly lower system detection limits. For target pesticide analysis in tobacco matrices, the Agilent 7000C Triple Quadrupole GC/MS/MS Analyzer for Pesticides in Tobacco comes with a turnkey method for 162 pesticides. It also includes an Agilent Pesticides and Environmental Pollutants MRM database (p/n G9250AA) of over 1,000 compounds, which makes the analytical task easy and productive.

The QuEChERS sample preparation technique [2,3,4] has been rapidly accepted worldwide for multipesticide residue analysis due to its attractive features, referred to as Quick, Easy, Cheap, Effective, Rugged, and Safe. QuEChERS extracts can be analyzed by GC combined with MS to determine a wide range of pesticide residues. Agilent Bond Elut QuEChERS Extraction Kits have demonstrated excellent recoveries for frequently used pesticides in different matrices [5]. However, tobacco extracts processed by QuEChERS are still very complex, containing various matrix residues such as high-boiling indigenous compounds.

QuEChERS extracts used in GC/MS/MS analysis can cause contamination and deterioration of the GC analytical column and MS ion source. This results in poor data quality due to poor peak shape, retention time shifting, and loss of responses for active analytes. These extracts also lead to shorter lifetime of GC analytical columns and frequent MS maintenance. To achieve low quantitation limits for pesticide analysis, it is necessary to use the best techniques and supplies to achieve reliable results, and to protect the analytical column and MS ion source.

Backflushing the GC column ensures that high-boiling compounds in the matrix do not pass through it, reducing column bleed, eliminating ghost peaks, and minimizing contamination of the mass spectrometer. Therefore, column backflushing can be beneficial for the analysis of tobacco extracts because it significantly reduces analysis time, and reduces both column head trimming and the frequency of MS ion source cleaning. Agilent capillary flow technology (CFT) makes column backflushing routine [6].

The Agilent Ultra Inert deactivation process significantly improves the inertness and robustness of wool liners. The wool surface is deactivated thoroughly. Ultra Inert splitless liners with wool have demonstrated excellent inertness in quantitative analysis of active and difficult pesticides in many matrices. Ultra Inert liners with wool also protect the sample flow path better, resulting in extended column lifetime and less frequent MS source maintenance [7].

This application note describes a study using midcolumn backflushing and the 7000C Triple Quadrupole GC/MS to measure 162 pesticide residues in tobacco. The pesticides were selected because of their presence in the CORESTA GRL list [1], and in the list of pesticides banned or recommended for tobacco cultivation in China.

Experimental

Tobacco matrix blanks, extracted using the QuEChERS method, were spiked with pesticide working solutions. The matrix-matched working calibration standards were then analyzed by GC/MS/MS using multiple reaction monitoring (MRM). A calibration curve from 0.01 to 2 mg/kg was used to evaluate linearity. Pesticide-free tobacco samples spiked with known concentrations of pesticide solutions were extracted by QuEChERS for recovery, repeatability, limit of detection (LOD), and limit of quantitation (LOQ) studies. In all studies, the unit of mg/kg was based on pesticide:tobacco (w:w), assuming the water content in the tobacco samples was zero.

Chemicals and reagents

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) was from J&K Scientific (Beijing, China). Toluene was from ANPEL Scientific Instrument (Shanghai, China). Water was from J. T. Baker. A set of pesticide stock solutions in acetonitrile (100 µg/mL) and the internal standard stock solution in acetonitrile (triphenyl phosphate, TPP), 1,000 µg/mL) were purchased from Ultra Scientific (North Kingstown, RI, USA).

Reagent solution preparation

The pesticide composite intermediate solution (2 µg/mL) was prepared by mixing and diluting the pesticide stock solutions with acetonitrile:toluene (2:1, v:v). The Internal Standard (ISTD) intermediate solution (20 µg/mL) was prepared by diluting the internal standard stock solution with acetonitrile:toluene (2:1, v/v).

Pesticide working solutions were used for preparation of matrix-matched standards. The working solutions contained ISTD at a constant concentration and pesticide analytes at appropriate concentration levels. Pesticide composite intermediate solution (2 µg/mL), ISTD intermediate solution (20 µg/mL), and acetonitrile:toluene (2:1, v:v) were used to prepare the working solutions.

Matrix-matched working calibration standards were derived from blank tobacco extracts as described in the sample preparation procedure. Appropriate pesticide working solutions were added to achieve matrix-matched working calibration standards from 0.01 to 2 mg/kg (pesticide:tobacco, w:w), assuming the water content in blank tobacco samples was zero.

Sample preparation

Preparation of tobacco extracts was based on the modified EN version of the QuEChERS method. Two grams of tobacco were extracted with 10 mL acetonitrile, diluted with 5 mL toluene, extracted with an Agilent Bond Elut QuEChERS Extraction Kit (p/n 5982-5650), and cleaned up using an Agilent Bond Elut QuEChERS Dispersive Kit (p/n 5982-5022). ISTD was added to the sample before extraction to control the entire analytical process. The QuEChERS procedure is shown below.

Weigh 2 g tobacco sample into a 50 mL tube
↓
Add 10 mL water, mix uniformly, and let stand for 10 minutes
↓
Add 10 mL ACN and ISDTs, vortex 2 minutes at 2,000 rpm
↓
Place the tube into a refrigerator at –18 °C for 10 minutes
↓
Add the salts, shake and vortex 2 minutes at 2,000 rpm
↓
Centrifuge at 4,000 rpm for 5 minutes
↓
Transfer 1.5 mL of supernatant to the dispersive SPE tube
↓
Vortex 2 minutes at 2,000 rpm
↓
Centrifuge at 4,000 rpm for 5 minutes
↓
Transfer the supernatant to a sample vial for analysis

Blank tobacco extracts were prepared in the same way as the sample treatment procedure, except there was no addition of the ISTD solution, and 1 mL final supernatant was blown to near dryness under a nitrogen stream.

Instrumentation

This study was performed on an Agilent 7890A GC coupled to an Agilent 7000C Triple Quadrupole GC/MS with an electron ionization (EI) source. The GC system was equipped with an Electronic Pneumatics Control (EPC), a Multimode Inlet (MMI), an Agilent 7693A Automatic Liquid Sampler, and a backflushing system based on a Purged Ultimate Union controlled by an AUX EPC module. Two Agilent J&W DB-5ms Ultra Inert GC 15 m columns were used to provide analyte separation and a highly inert flow path into the detector. Agilent MassHunter Software was used for instrument control and for qualitative and quantitative data analysis. Retention time locking eliminated the need to adjust time segment windows of MRM groups [8]. The run time was 40.5 minutes with an extra 5 minutes for backflushing.

The Agilent MRM Database was used to help build the MS acquisition method for the target analytes by selecting the MRM transitions with minimum matrix interferences and maximum responses. For each pesticide, two MRM transitions were selected for quantitation and qualification, and the collision energy was optimized. However, different transitions might be used for quantitation in different tobacco matrices to minimize matrix effects. Therefore, it is critical to review the data in matrix before setting up a quantitation method.

Table 1 lists the instrument parameters used in this study. Table 2 shows the consumable supplies, and Table 3 the pesticides in alphabetical order with their quant and qual transitions, and the collision energies for each.

Table 1. Instrumentation and analytical conditions.

GC conditions	
Column 1	Agilent J&W DB-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 122-5512UI), configured from MMI to AUX EPC
Column 2	Agilent J&W DB-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 122-5512UI), configured from AUX EPC to vacuum
Carrier gas	Helium
Injection mode	Splitless
Injection volume	1 µL
Solvent washes	**Pre-injection** 2 × solvent A, acetonitrile, max volume 2 × solvent B, toluene, max volume **Post-injection** 5 × solvent A, acetonitrile, max volume 5 × solvent B, toluene, max volume
Sample wash	1 × 3 µL
Sample pumps	3
Injection speed	Fast
MMI temperature program	70 °C for 0 min, then 240 °C/min to 280 °C until the end of the analysis
Purge flow to split vent	50 mL/min at 1.25 min
Gas saver	On 20 mL/min after 10 min
Septum purge flow	3 mL/min
Oven temperature program	60 °C for 1 min, then 40 °C/min to 120 °C, then 5 °C/min to 310 °C
Column 1 flow	1 mL/min
Column 2 flow	1.2 mL/min
Retention time locking	Chlorpyrifos-methyl locked at 18.700 min
Run time	40.5 min
Post run	5 min at 310 °C, AUX EPC pressure 50 psi, inlet pressure 2 psi
MS conditions	
MS source	EI, –70 eV
Source temperature	300 °C
Quadrupole temperature	180 °C
Transfer line temperature	280 °C
Solvent delay	3.75 min
Helium quench gas	2.25 mL/min
Nitrogen collision gas	1.5 mL/min
Acquisition mode	Multiple reaction monitoring
MS1/MS2 resolution	Wide
Time segments	Refer to Table 3
Acquisition parameters	Refer to Table 3

Table 2. Consumable supplies.

Vials	Amber, write-on spot, 100/pk (p/n 5182-0716)
Vial caps	Blue, screw cap, 100/pk (p/n 5182-0717)
Vial inserts	150 µL glass with polymer feet, 100/pk (p/n 5183-2088)
Septa	Advanced green, 50/pk (p/n 5183-4759)
Inlet liners	4 mm id liner, UI, splitless, single taper, glass wool (p/n 5190-2293)
Agilent Bond Elut QuEChERS Extraction Kits EN	p/n 5982-5650
Agilent Bond Elut QuEChERS Dispersive Kits	p/n 5982-5022

Table 3. Quantifier and qualifier MRM transitions for target pesticides*.

	Quantifier		Qualifier	
Compound	Transition	CE	Transition	CE
3-Hydroxycarbofuran	180.0 → 137.0	15	137.0 → 107.0	15
Acephate	136.0 → 42.0	5	142.0 → 96.0	5
Acetamiprid	152.0 → 116.1	15	126.0 → 73.0	25
Acibenzolar-S-methyl	135.0 → 63.1	20	182.0 → 181.1	5
Alachlor	237.0 → 160.1	5	269.0 → 188.0	5
Aldrin	262.9 → 192.9	35	262.9 → 190.9	35
alpha-Endosulfan	262.8 → 192.9	30	195.0 → 160.0	10
Atrazine	214.9 → 58.1	10	214.9 → 200.2	5
Azinphos-ethyl	160.0 → 77.1	20	160.0 → 77.0	20
Azinphos-methyl	160.0 → 77.1	20	132.0 → 51.0	30
Azoxystrobin	344.1 → 171.9	40	344.1 → 329.0	15
Benalaxyl	266.0 → 148.1	5	233.9 → 146.0	20
Benfluralin	292.0 → 264.0	5	292.0 → 206.0	10
Benfuracarb	190.0 → 102.0	10	190.0 → 74.0	20
beta-Endosulfan	241.0 → 206.0	25	206.9 → 172.0	15
Bifenthrin	181.2 → 165.2	25	181.2 → 166.2	10
Bitertanol	170.1 → 141.1	20	170.1 → 115.0	40
Bromacil	205.0 → 188.0	15	207.0 → 190.0	15
Bromophos	330.8 → 315.8	15	328.8 → 313.8	15
Butralin	266.0 → 220.2	10	266.0 → 174.2	20
Cadusafos	158.8 → 97.0	15	157.9 → 96.9	15
Captafol	310.8 → 78.8	15	150.0 → 71.9	5
Captan	116.9 → 82.0	30	149.0 → 70.0	15
Carbaryl	144.0 → 115.1	20	144.0 → 116.1	10
Carbofuran	221.0 → 164.0	10	164.0 → 103.0	25
Carbosulfan	118.0 → 76.0	5	160.0 → 62.0	20
Chinomethionate (oxythioquinox)	233.9 → 206.1	10	233.9 → 148.1	25
Chlorantraniliprole	278.0 → 249.0	25	278.0 → 215.0	30
Chlordane (*cis*-)	372.9 → 265.9	20	271.9 → 236.9	15
Chlordane (*trans*-)	271.7 → 236.9	15	372.8 → 265.8	15
Chlordimeform	151.9 → 117.1	10	180.9 → 140.0	15
Chlorfenvinphos	266.9 → 159.1	15	268.9 → 161.0	15
Chlornitrofen	316.6 → 286.6	15	316.6 → 195.6	25
Chlorobenzilate	251.1 → 139.1	15	139.1 → 75.1	30
Chlorothalonil	263.8 → 133.0	40	264.0 → 168.0	25
Chlorpyrifos	196.9 → 169.0	15	198.9 → 171.0	15
Chlorpyrifos-methyl	285.9 → 92.9	20	124.9 → 47.0	15
Chlorthal-dimethyl (DCPA)	300.9 → 223.0	25	298.9 → 221.0	25
Clomazone	125.0 → 89.0	15	204.0 → 78.0	30
Cyfluthrin	226.0 → 206.0	15	162.9 → 127.0	5
Cyhalothrin (lambda)	208.0 → 181.0	5	181.1 → 152.0	25
Cypermethrin	165.1 → 127.1	5	163.1 → 127.1	5
Dazomet	161.9 → 89.0	5	89.0 → 46.0	15
DBCP	157.0 → 75.0	5	155.0 → 75.0	5
Deltamethrin	252.9 → 93.0	15	253.0 → 174.0	5
Demeton-O	88.0 → 60.0	5	171.0 → 115.0	10
Demeton-S	88.0 → 60.0	5	126.0 → 65.0	10
Demeton-S-methyl	88.0 → 60.0	5	142.0 → 78.9	10
Demeton-S-methyl sulfone	169.1 → 109.0	15	169.1 → 125.1	5
Diazinon	304.0 → 179.0	15	137.1 → 84.0	10
Dichlorvos	184.9 → 93.0	10	185.0 → 109.0	15
Dicloran	160.1 → 124.1	10	206.1 → 176.0	10
Dieldrin	262.9 → 193.0	35	262.9 → 191.0	35
Difenoconazole	322.8 → 264.8	15	264.9 → 202.0	20
Diflubenzuron	153.0 → 125.0	15	153.0 → 91.0	30
Dimefox	110.0 → 47.0	35	153.0 → 110.0	10
Dimetachlone	187.0 → 152.0	5	243.0 → 187.0	10
Dimethoate	86.9 → 46.0	15	92.9 → 63.0	10
Dimethomorph (E)	301.0 → 139.0	15	300.9 → 165.0	10
Dimethomorph (Z)	301.0 → 139.0	15	300.9 → 165.0	10
Diphenamid	167.1 → 165.1	20	167.1 → 152.1	15
Disulfoton	88.0 → 60.0	5	142.0 → 81.0	10
Disulfoton sulfone	213.0 → 96.9	15	152.9 → 97.0	10
Disulfoton sulfoxide	97.0 → 65.0	20	212.0 → 97.0	30
Endosulfan-sulphate	271.9 → 237.0	15	273.8 → 238.9	15
Endrin	262.8 → 193.0	35	263.0 → 228.0	20
EPN	157.0 → 110.0	15	141.0 → 77.1	15
Ethion	230.9 → 129.0	20	231.0 → 175.0	10
Ethoprophos	157.9 → 97.0	15	157.9 → 114.0	5
Famoxadone	329.9 → 223.9	10	330.0 → 224.0	5
Fenamiphos (phenamiphos)	303.0 → 154.0	15	303.0 → 180.0	20
Fenamiphos sulfone	319.8 → 292.0	10	291.8 → 214.0	10
Fenamiphos-sulfoxide	304.0 → 196.0	5	304.0 → 122.0	15
Fenchlorphos (Ronnel)	285.0 → 269.9	15	286.9 → 272.0	15
Fenitrothion	277.0 → 260.1	5	277.0 → 109.0	15
Fensulfothion	291.8 → 156.0	15	292.8 → 96.8	20
Fenthion	278.0 → 169.0	15	278.0 → 109.0	15
Fenthion sulfone	309.9 → 105.0	10	124.9 → 47.0	10
Fenthion sulfoxide	125.0 → 47.0	10	278.0 → 109.0	15
Fenvalerate	224.9 → 119.0	15	167.0 → 125.1	5
Flucythrinate	156.9 → 107.1	15	198.9 → 107.0	25
Flumetralin	143.0 → 107.1	20	157.0 → 109.0	25
Folpet	260.0 → 130.0	15	261.8 → 130.1	15
Fonofos	245.9 → 137.0	5	245.9 → 109.0	15
Formothion	124.9 → 47.0	15	170.0 → 93.0	5
gamma-HCH (lindane)	219.0 → 183.0	5	181.0 → 145.0	15
HCH (*alpha*-)	216.9 → 181.0	5	219.0 → 183.0	5
HCH (*beta*-)	218.9 → 183.1	5	219.0 → 183.0	5
HCH (*delta*-)	217.0 → 181.1	5	219.0 → 183.1	5
Heptachlor	271.7 → 236.9	15	273.7 → 238.9	15
Heptachlor epoxides (*cis*-)	352.8 → 262.9	15	354.8 → 264.9	15
Heptachlor epoxides (*trans*-)	182.9 → 118.9	25	289.0 → 219.0	30
Heptenophos	124.0 → 89.0	10	124.0 → 63.0	35
Hexachlorobenzene	283.8 → 213.9	30	281.8 → 211.9	30
Indoxacarb	202.9 → 134.0	15	203.0 → 78.0	30
Iprobenfos	245.9 → 91.0	15	203.9 → 91.0	5
Iprodione	313.8 → 55.9	20	313.8 → 244.9	10
Isazophos	257.0 → 119.0	15	257.0 → 162.0	5
Isopropalin	264.0 → 222.2	5	238.0 → 165.2	10

*Note: for analytes with multiple artifacts, the one with the highest response was analyzed and quantified.

Table 3. Quantifier and qualifier MRM transitions for target pesticides*.

	Quantifier		Qualifier			Quantifier		Qualifier	
Compound	**Transition**	**CE**	**Transition**	**CE**	**Compound**	**Transition**	**CE**	**Transition**	**CE**
Isoprothiolane	162.1 → 85.0	20	189.1 → 89.0	20	Pendimethalin (penoxaline)	251.8 → 162.2	10	251.8 → 161.1	15
Leptophos	377.0 → 362.0	20	376.8 → 361.8	20	Permethrin	163.0 → 127.0	5	183.1 → 165.1	10
Malathion	172.9 → 99.0	15	126.9 → 99.0	5	Phorate	121.0 → 47.0	30	260.0 → 75.0	10
Metalaxyl	234.0 → 146.1	20	234.0 → 174.1	10	Phosalone	182.0 → 75.1	30	182.0 → 111.0	15
Methamidophos	141.0 → 64.0	20	141.0 → 95.0	5	Phosphamidon (E)	264.0 → 127.0	15	264.0 → 72.0	10
Methidathion	144.9 → 85.0	5	144.9 → 58.1	15	Phosphamidon (Z)	264.0 → 127.0	15	192.9 → 127.0	5
Methiocarb	168.0 → 109.1	15	168.0 → 91.0	30	Piperonyl butoxide	176.1 → 103.1	25	176.1 → 131.1	15
Methiocarb sulfone	200.0 → 121.0	15	185.0 → 121.0	5	Pirimicarb	238.0 → 166.2	10	166.0 → 55.1	20
Methomyl	105.0 → 88.0	5	105.0 → 58.0	10	Pirimiphos-methyl	290.0 → 125.0	20	232.9 → 151.0	5
Methoprene	153.0 → 111.1	5	153.0 → 83.0	20	Profenofos	338.8 → 268.7	15	207.9 → 63.0	30
Methoxychlor	227.0 → 169.1	25	227.0 → 141.1	40	Propoxur	110.0 → 63.0	25	110.0 → 64.0	15
Metolachlor	238.0 → 162.2	10	162.2 → 133.2	15	Prothiofos	308.9 → 238.9	15	266.9 → 239.0	5
Mevinphos	127.0 → 95.0	15	192.0 → 127.0	10	Pyrazophos	221.0 → 193.1	10	232.0 → 204.1	10
Mexacarbate	165.1 → 134.0	10	165.1 → 150.0	15	Quinalphos	298.0 → 156.0	20	157.0 → 102.0	30
Mirex	271.8 → 236.8	15	273.8 → 238.8	15	Quizalofop-*p*-ethyl	371.8 → 298.9	10	163.0 → 136.0	10
Monocrotophos	192.0 → 127.1	10	127.1 → 95.0	15	Schradan	153.1 → 46.1	15	199.0 → 92.0	5
Myclobutanil	179.0 → 125.1	10	150.0 → 123.0	15	Tefluthrin	177.1 → 87.0	30	177.1 → 127.1	15
Naled	144.9 → 109.0	15	184.9 → 93.0	15	Teflubenzuron	197.0 → 135.0	30	197.0 → 142.0	25
Napropamide	271.0 → 72.1	15	128.0 → 72.1	5	Terbufos	230.9 → 129.0	20	230.9 → 175.0	10
Nitrofen	202.0 → 139.1	20	282.9 → 253.0	10	Terbufos sulfone	264.0 → 97.0	25	199.0 → 97.0	20
o,p'-DDD	235.0 → 165.2	20	237.0 → 165.2	20	Tetrachlorvinphos	330.8 → 108.9	15	328.8 → 108.9	15
o,p'-DDE	246.0 → 176.2	30	248.0 → 176.2	30	Tetradifon	158.9 → 111.0	20	353.8 → 226.8	10
o,p'-DDT	235.0 → 165.0	20	237.0 → 165.0	20	Thiamethoxam	212.0 → 139.0	15	212.0 → 125.0	10
Omethoate	155.9 → 110.0	5	155.9 → 79.0	20	Thionazin	143.0 → 79.0	10	175.0 → 79.0	10
Oxadixyl	163.0 → 132.1	5	232.9 → 146.1	10	Triadimefon	208.0 → 181.1	5	208.0 → 111.0	20
Oxamyl	98.0 → 58.0	10	145.0 → 71.9	20	Triadimenol	168.0 → 70.0	10	128.0 → 65.0	25
p,p'-DDD	235.0 → 165.0	20	237.0 → 165.0	20	Triazophos	257.0 → 162.1	5	161.2 → 134.2	5
p,p'-DDE	315.8 → 246.0	15	246.1 → 176.2	30	Trichlorfon	145.0 → 109.0	12	109.0 → 79.0	10
p,p'-DDT	235.0 → 165.0	20	237.0 → 165.0	20	Triflumuron	139.0 → 75.0	30	139.0 → 111.0	15
Parathion	290.9 → 109.0	10	138.9 → 109.0	5	Trifluralin	305.9 → 264.0	5	290.0 → 248.0	5
Parathion-methyl	262.9 → 109.0	10	232.9 → 109.0	10	Uniconazole	234.1 → 137.0	15	234.1 → 165.1	10
Penconazole	248.0 → 192.1	15	248.0 → 157.1	25	Vamidothion	141.9 → 78.9	10	145.0 → 87.0	5

Note: for analytes with multiple artifacts, the one with the highest response was analyzed and quantified.

Results and Discussion

Recovery

Table 4 shows the mean recoveries of three repetitive tobacco samples at 0.05 and 0.5 mg/kg, respectively. For both concentration levels, 95% of the pesticides were in the range of 70 to 120%, showing excellent recoveries. The mean recoveries of 70 to 120% and RSD ≤ 20% represent widely acceptable validation criteria in pesticide residue analysis, but other criteria could be used and justified depending on the purpose of the analysis. For example, the Pesticide Data Program (PDP) requires mean recoveries of 50 to 150% for methods used for analysis of PDP samples [9], because the main aim of PDP is to provide exposure data, and ideally include as many pesticides as possible in multiresidue methods.

If they are consistent (RSD ≤ 20%), some compounds with recoveries outside 70 to 120% can still be analyzed by QuEChERS, but may require special consideration. Compounds such as captan, chlorothalonil, diflubenzuron, and folpet are base-sensitive and unstable even in acetonitrile. They tend to degrade in the presence of basic compounds (at higher pH), and often present issues in terms of recovery from the matrix and precision during analysis. Although not used in this study, the evaluation of their corresponding internal standards such as captan-d6 and folpet-d4 are recommended [10] to control recovery and ensure reliable results, especially for longer batches in which the number of injections exceeds 40. Compounds such as benfuracarb and carbosulfan are acid-sensitive and degrade at lower pH.

Table 4. Method validation data: linearity, recovery (n = 3), %RSD (n = 3), LOD, and LOQ of 162 pesticides.

			Recovery and %RSD					
	Linearity		0.05 mg/kg		0.5 mg/kg			
Pesticide	Linear range (mg/kg)	R^2	Recovery (%)	%RSD	Recovery (%)	%RSD	LOD (mg/kg)	LOQ (mg/kg)
3-Hydroxycarbofuran	0.01-2	0.9958	95.26	11.98	106.21	6.17	0.0416	0.1388
Acephate	0.01-2	0.9973	78.95	9.89	76.12	4.11	0.0036	0.0121
Acetamiprid	0.01-2	0.9985	101.3	2.34	107.03	2.11	0.0074	0.0246
Acibenzolar-S-methyl	0.01-2	0.9976	88.61	0.48	98.16	1.65	0.0014	0.0045
Alachlor	0.01-2	0.9984	99.29	3.2	100.68	1.86	0.0015	0.0052
Aldrin	0.01-2	0.9997	92.71	0.93	105.2	0.86	0.002	0.0068
alpha-Endosulfan	0.01-2	0.9993	101.24	4.88	108.07	2.46	0.0026	0.0088
Azinphos-ethyl	0.01-2	0.9925	92.8	2.32	94.73	2.32	0.0018	0.0059
Azinphos-methyl	0.01-2	0.9942	103.94	5.11	88.6	0.57	0.0213	0.0711
Azoxystrobin	0.01-2	0.9977	104.3	6.05	108.05	1.68	0.0037	0.0125
Benalaxyl	0.01-2	0.9996	104.12	2.07	106.13	2.77	0.001	0.0034
Benfluralin	0.01-2	0.9796	86.6	3.81	85.13	2.57	0.0023	0.0077
Benfuracarb	0.01-2	0.9990	78.45	2.82	78.57	3.09	0.0023	0.0077
beta-Endosulfan	0.01-2	0.9993	103.62	5.35	105.88	1.83	0.0067	0.0223
Bifenthrin	0.01-2	0.9999	103.37	1.28	108.8	1.61	0.0026	0.0088
Bitertanol	0.01-2	0.9994	103.58	2.89	106.16	1.45	0.0027	0.0089
Bromacil	0.01-2	0.9992	96.42	1.63	101.98	1.86	0.0013	0.0044
Bromophos	0.01-2	0.9990	95.63	1.96	102.49	1.6	0.0012	0.0041
Butralin	0.01-2	0.9764	86.01	2.22	84.08	2.05	0.0025	0.0083
Cadusafos	0.01-2	0.9990	94.92	5.19	100.05	2.46	0.0026	0.0086
Captafol	0.01-2	0.9991	94.37	22.53	74.16	10.38	0.0222	0.0739
Captan	0.01-2	0.9954	77.23	3.74	79.66	9.92	0.0061	0.0204
Carbaryl	0.01-2	0.9991	98.84	0.78	102.09	2.79	0.0032	0.0107
Carbofuran	0.01-2	0.9967	113.25	13.81	107.89	1.65	0.0134	0.0446
Carbosulfan	0.01-2	0.9980	83.01	5.14	98.27	0.6	0.0018	0.0059
Chinomethionate (oxythioquinox)	0.01-2	0.9989	63.97	5.88	58.37	2.49	0.0017	0.0056
Chlorantraniliprole	0.01-2	0.9982	129.44	2.96	123.13	3.23	0.004	0.0132
Chlordane (*cis*-)	0.01-2	0.9994	94.74	7.3	105.68	2.6	0.0031	0.0103
Chlordane (*trans*-)	0.01-2	0.9995	97.11	2.41	105.74	1.86	0.0027	0.0091
Chlordimeform	0.01-2	0.9996	92.57	2.85	93.24	1.97	0.0026	0.0086
Chlorfenvinphos	0.01-2	0.9989	101.32	3.62	104.28	1.26	0.0011	0.0037
Chlornitrofen	0.01-2	0.9886	92.29	1.41	93.14	3.44	0.0039	0.0129
Chlorobenzilate	0.01-2	0.9996	101.61	1.95	107.34	1.65	0.0011	0.0037
Chlorothalonil	0.01-2	0.9958	31.84	15.52	46.19	3.11	0.0019	0.0065
Chlorpyrifos	0.01-2	0.9992	95.06	6.73	105.14	1.95	0.0025	0.0085
Chlorpyrifos-methyl	0.01-2	0.9982	94.51	3.18	100.01	1.85	0.0023	0.0076
Chlorthal-dimethyl (DCPA)	0.01-2	0.9996	99.72	3.62	106.3	1.58	0.0008	0.0027
Clomazone	0.01-2	0.9995	97.78	2.71	100.83	3.97	0.0014	0.0045
Cyfluthrin	0.01-2	0.9940	99.34	4.3	99.79	2.14	0.0014	0.0047
Cyhalothrin (*lambda*)	0.01-2	0.9931	96.48	3.61	95.36	0.57	0.0012	0.004
Cypermethrin	0.01-2	0.9927	103.83	5.85	101.93	1.04	0.0025	0.0083
Dazomet	0.01-2	0.9971	78.53	7.59	88.67	2.76	0.003	0.0099
DBCP	0.01-2	0.9999	86.41	0.64	93.17	6.56	0.0019	0.0062
Deltamethrin	0.01-2	0.9864	95.48	6.67	91.64	2.23	0.003	0.0101
Demeton-O	0.01-2	0.9991	107.06	10.43	101.67	2.54	0.0017	0.0057
Demeton-S	0.01-2	0.9980	94.01	6.46	98.75	2.47	0.002	0.0065
Demeton-S-methyl	0.01-2	0.9979	93.46	6.69	99.2	2.36	0.004	0.0133
Demeton-S-methyl sulfone	0.01-2	0.9945	90.44	4.22	86.32	3.97	0.0031	0.0103
Diazinon	0.01-2	0.9992	95.75	3.04	100.53	3.04	0.003	0.0099
Dichlorvos	0.01-2	0.9999	112.03	5.74	122.71	3.44	0.0037	0.0122

	Linearity		Recovery and %RSD					
			0.05 mg/kg		0.5 mg/kg			
Pesticide	Linear range (mg/kg)	R^2	Recovery (%)	%RSD	Recovery (%)	%RSD	LOD (mg/kg)	LOQ (mg/kg)
Dicloran	0.01-2	0.9950	93.75	2.11	95.61	4.68	0.0028	0.0092
Dieldrin	0.01-2	0.9998	105.82	5.78	108.27	2.73	0.0043	0.0142
Difenoconazole	0.01-2	0.9985	105.12	2.14	108.8	1.27	0.0034	0.0115
Dimefox	0.01-2	0.9997	90.95	8.55	94.64	5.17	0.0036	0.0121
Dimetachlone	0.01-2	0.9998	99.98	5.29	105.32	1.69	0.003	0.01
Dimethoate	0.01-2	0.9960	97.04	5.59	98.46	0.61	0.003	0.0101
Dimethomorph (E)	0.01-2	0.9990	104.55	4.02	109.43	1.11	0.0017	0.0055
Dimethomorph (Z)	0.01-2	0.9997	105.95	3.4	113.19	2.29	0.0028	0.0093
Diphenamid	0.01-2	0.9996	101.55	4.29	105.3	1.21	0.0011	0.0036
Disulfoton	0.01-2	0.9984	92.8	1.86	99.38	2.75	0.0023	0.0075
Disulfoton sulfone	0.01-2	0.9965	94.79	5.19	99.66	0.95	0.0026	0.0088
Disulfoton sulfoxide	0.01-2	0.9976	105.45	8.03	104.96	8.06	0.003	0.01
Endosulfan-sulphate	0.01-2	0.9995	102.74	2.37	106.35	1.13	0.0009	0.0031
Endrin	0.01-2	0.9994	94.83	3.33	105.86	1.11	0.0039	0.0129
EPN	0.01-2	0.9946	96.29	3.99	97.99	2.26	0.0014	0.0048
Ethion	0.01-2	0.9959	97.75	4.41	101.84	2.27	0.0016	0.0052
Ethoprophos	0.01-2	0.9989	93.44	5.69	100.76	1.57	0.0033	0.0108
Famoxadone	0.01-2	0.9845	96.3	7.56	94.2	1.15	0.0039	0.0131
Fenamiphos (phenamiphos)	0.01-2	0.9991	99.23	3.11	102.83	1.86	0.0023	0.0075
Fenamiphos sulfone	0.01-2	0.9979	101.7	3.25	102.54	1.47	0.0013	0.0045
Fenamiphos-sulfoxide	0.01-2	0.9840	97.96	6.87	86.91	0.81	0.002	0.0067
Fenchlorphos (Ronnel)	0.01-2	0.9990	97.07	4.74	104.12	0.96	0.002	0.0065
Fenitrothion	0.01-2	0.9804	86.01	3.82	86.01	0.74	0.0026	0.0085
Fensulfothion	0.01-2	0.9874	113.76	2.77	120.81	3.12	0.0028	0.0094
Fenthion	0.01-2	0.9994	97.7	5.98	103.19	0.1	0.0024	0.0078
Fenthion sulfone	0.01-2	0.9979	93.67	6.96	97.65	2.82	0.0031	0.0102
Fenthion sulfoxide	0.01-2	0.9976	95.04	4.15	96.24	1.56	0.002	0.0066
Fenvalerate	0.01-2	0.9878	112.17	1.57	95.4	2.55	0.0072	0.0241
Flucythrinate	0.01-2	0.9959	106.16	2.37	106.38	2.89	0.0009	0.0031
Flumetralin	0.01-2	0.9904	90.29	5.57	93.81	1.97	0.0023	0.0076
Folpet	0.01-2	0.9906	75.72	5.77	71.03	8.01	0.0025	0.0082
Fonofos	0.01-2	0.9985	93.59	3.44	98.78	2.8	0.0017	0.0056
Formothion	0.01-2	0.9971	86.49	2.55	87.42	3.52	0.0029	0.0098
gamma-HCH (lindane)	0.01-2	0.9988	93.99	1.46	96.48	4.32	0.0013	0.0043
HCH (*alpha*-)	0.01-2	0.9991	94.48	2.64	100.32	3.89	0.0015	0.0049
HCH (*beta*-)	0.01-2	0.9981	97.69	3.91	98.73	3.95	0.0012	0.0039
HCH (*delta*-)	0.01-2	0.9990	96.07	3.67	97.4	4.18	0.0013	0.0043
Heptachlor	0.01-2	0.9967	89.21	3.27	98.28	2.77	0.0013	0.0043
Heptachlor epoxides (*cis*-)	0.01-2	0.9993	95.93	3.73	105.75	2.06	0.002	0.0066
Heptachlor epoxides (*trans*-)	0.01-2	0.9996	104.44	6.65	106.73	2.91	0.0037	0.0125
Heptenophos	0.01-2	0.9989	93.56	5.38	99.32	2.22	0.0029	0.0098
Hexachlorobenzene	0.01-2	0.9997	83.24	2.29	95.74	3.13	0.0015	0.0051
Indoxacarb	0.01-2	0.9998	106.45	3.22	115.42	1.11	0.003	0.0099
Iprobenfos	0.01-2	0.9968	96.43	3.84	100.83	1.52	0.0035	0.0117
Iprodione	0.01-2	0.9999	99.45	5.51	105.61	2.37	0.0025	0.0085
Isazophos	0.01-2	0.9988	97.65	2.42	98.61	5.92	0.0029	0.0096
Isopropalin	0.01-2	0.9822	87.37	1.75	87.92	1.55	0.0024	0.0081
Isoprothiolane	0.01-2	0.9996	101.5	1.29	106.6	1.06	0.0009	0.0029
Leptophos	0.01-2	0.9991	96.11	3.87	104	1.77	0.0016	0.0052
Malathion	0.01-2	0.9976	96.68	4.77	100.62	1.21	0.0021	0.007
Metalaxyl	0.01-2	0.9997	99.25	4.16	105.66	1.52	0.0026	0.0087

	Linearity		Recovery and %RSD					
			0.05 mg/kg		0.5 mg/kg			
Pesticide	Linear range (mg/kg)	R^2	Recovery (%)	%RSD	Recovery (%)	%RSD	LOD (mg/kg)	LOQ (mg/kg)
Methamidophos	0.01-2	0.9985	64.8	9.05	72.27	3.05	0.004	0.0134
Methidathion	0.01-2	0.9980	94.26	2.82	98.29	1.07	0.0023	0.0075
Methiocarb	0.01-2	0.9993	97.78	4.25	102.84	1.89	0.0017	0.0057
Methiocarb sulfone	0.01-2	0.9973	92.5	2.96	100.65	1.07	0.0035	0.0118
Methomyl	0.01-2	0.9970	120.17	10.64	106.37	10.74	0.006	0.0201
Methoprene	0.01-2	0.9988	107.7	10.08	104.43	3.23	0.0179	0.0595
Methoxychlor	0.01-2	0.9970	93.03	1.39	94.25	5.39	0.0022	0.0072
Metolachlor	0.01-2	0.9991	97.92	2.1	103.27	1.29	0.0009	0.003
Mevinphos	0.01-2	0.9992	95.16	4.22	97.9	1.37	0.0058	0.0195
Mexacarbate	0.01-2	0.9991	99.65	4.42	101.16	3.76	0.0021	0.007
Mirex	0.01-2	0.9995	88.83	1.75	103.68	1.92	0.001	0.0032
Monocrotophos	0.01-2	0.9946	88	10.24	90.02	2.7	0.0052	0.0172
Myclobutanil	0.01-2	0.9997	103.08	1	106.29	1.24	0.0013	0.0044
Naled	0.01-2	0.9872	27.35	2.48	15.82	18.06	0.0019	0.0062
Napropamide	0.01-2	0.9995	101.99	1.08	105.75	1.08	0.0025	0.0083
Nitrofen	0.01-2	0.9924	93.4	2.75	94.42	1.19	0.0028	0.0095
o,p'-DDD	0.01-2	0.9997	101.75	0.8	110.48	1.03	0.001	0.0034
o,p'-DDE	0.01-2	0.9997	95.92	1.07	107.07	0.81	0.0015	0.0051
o,p'-DDT	0.01-2	0.9975	95.75	1.18	100	4.94	0.0021	0.007
Omethoate	0.01-2	0.9961	83.31	11.12	82.39	4.61	0.0037	0.0123
Oxadixyl	0.01-2	0.9995	106.24	3.18	106.29	1.77	0.0015	0.0051
Oxamyl	0.01-2	0.9971	107.18	8.43	96.25	5.86	0.0207	0.0689
p,p'-DDD	0.01-2	0.9991	105.79	2.1	109.09	1.4	0.001	0.0032
p,p'-DDE	0.01-2	0.9990	158.52	6.65	111.94	2.2	0.0105	0.035
p,p'-DDT	0.01-2	0.9967	100.53	3.45	95.5	6.79	0.0036	0.0119
Parathion	0.01-2	0.9754	88.29	3.98	86.33	1.52	0.003	0.0099
Parathion-methyl	0.01-2	0.9863	87.19	4.1	88.47	3.18	0.0028	0.0094
Penconazole	0.01-2	0.9994	100.09	3.94	103.44	0.47	0.0018	0.0059
Pendimethalin (penoxaline)	0.01-2	0.9769	82.87	3.16	83.8	1.8	0.003	0.0099
Permethrin	0.01-2	0.9993	102.01	0.87	107.26	1.24	0.002	0.0066
Phorate	0.01-2	0.9978	94.43	3.97	98.14	2.82	0.0029	0.0095
Phosalone	0.01-2	0.9986	96.62	2.4	100.12	1.4	0.0012	0.0039
Phosphamidon (E)	0.01-2	0.9971	93.1	13.39	98.58	3.89	0.0012	0.0041
Phosphamidon (Z)	0.01-2	0.9972	97.94	4.64	98.44	2.45	0.0015	0.005
Piperonyl butoxide	0.01-2	0.9996	110.28	1.59	106.4	2.06	0.0023	0.0075
Pirimicarb	0.01-2	0.9997	101.58	1.97	103.44	1.41	0.0014	0.0047
Pirimiphos-methyl	0.01-2	0.9981	95.16	4.76	101.25	1.21	0.0017	0.0058
Profenofos	0.01-2	0.9988	98.56	2.31	104.9	1.83	0.0024	0.0082
Propoxur	0.01-2	0.9993	97.23	4.97	103.62	1.99	0.0026	0.0087
Prothiofos	0.01-2	0.9989	97.98	1.05	103.32	1.17	0.0016	0.0052
Pyrazophos	0.01-2	0.9970	99.63	1.98	100.77	1.4	0.0015	0.005
Quinalphos	0.01-2	0.9984	99.95	3.3	101.18	3.7	0.0041	0.0138
Quizalofop-*p*-ethyl	0.01-2	0.9988	102.46	4.23	107.96	1.99	0.0014	0.0048
Schradan	0.01-2	0.9939	94.61	7.7	85.95	3.14	0.0023	0.0076
Tefluthrin	0.01-2	0.9996	101.33	1.27	105.38	3.87	0.0014	0.0048
Teflubenzuron	0.01-2	0.9996	95.89	6.64	99.87	5.37	0.0016	0.0052
Terbufos	0.01-2	0.9965	92.01	5.13	97.03	3.05	0.0024	0.0079
Terbufos sulfone	0.01-2	0.9972	91.68	4.51	100.41	2.53	0.0013	0.0042
Tetrachlorvinphos	0.01-2	0.9989	97.53	1.72	99.85	1.16	0.0025	0.0082
Tetradifon	0.01-2	0.9997	103.63	2.25	106.68	2.12	0.0008	0.0028
Thiamethoxam	0.01-2	0.9986	101.04	3.29	100.03	1.66	0.0018	0.006

Pesticide	Linearity		Recovery and %RSD 0.05 mg/kg		0.5 mg/kg		LOD (mg/kg)	LOQ (mg/kg)
	Linear range (mg/kg)	R^2	Recovery (%)	%RSD	Recovery (%)	%RSD		
Thionazin	0.01-2	0.9988	94.57	5.34	100.92	2.39	0.0028	0.0094
Triadimefon	0.01-2	0.9994	97.77	3.36	103.18	2.13	0.002	0.0066
Triadimenol	0.01-2	0.9998	96.31	4.96	104.37	0.66	0.0017	0.0058
Triazophos	0.01-2	0.9976	101.33	1.78	101.32	2.38	0.0022	0.0073
Trichlorfon	0.01-2	0.9970	86.41	12	84.19	5.46	0.0042	0.0138
Triflumuron	0.01-2	0.9993	96.79	4.56	103.99	1.55	0.0031	0.0103
Trifluralin	0.01-2	0.9887	91.83	6.06	92.37	2.27	0.0034	0.0113
Uniconazole	0.01-2	0.9995	102.08	2.6	104.09	0.82	0.0025	0.0084
Vamidothion	0.01-2	0.9944	86.29	11.05	85.34	2.68	0.0024	0.0081

Backflushing the column

The GC/MS/MS system used a Purged Ultimate Union (PUU), and its configuration is shown in Figure 1. Backflushing was done for 5 minutes after the run by raising the pressure at the PUU and lowering the inlet pressure. This reversed the flow through the column and purged high-boiling matrix components from the head of the column, out through the inlet's split vent. With the PUU installed, inlet and GC column maintenance is possible without venting the mass spectrometer. Figure 2 shows that 5 minutes of backflushing cleaned the analytical columns and reduced the cycle time for target analytes in tobacco extracts.

During the course of this study, approximately 200+ 1 µL injections of concentrated tobacco extracts were made into the GC/MS/MS system with no evidence of column or MS performance problems, as shown in Figure 3.

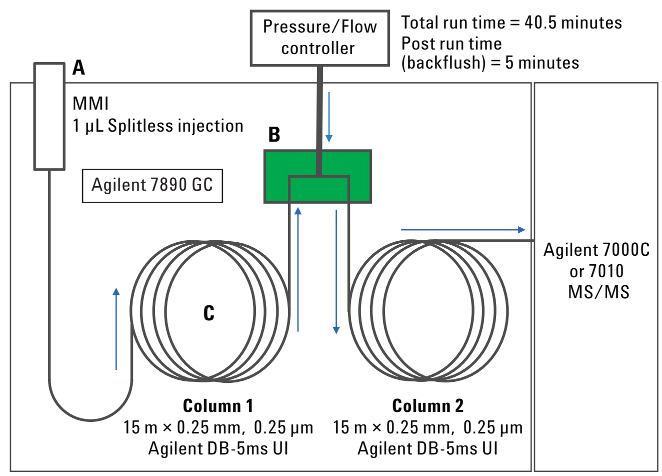

Figure 1. Hardware diagram of the Agilent Tobacco Analyzer. The GC/MS/MS system used for MRM analysis was configured with A) Multimode Inlet, B) Purged Ultimate Union, and C) two Agilent J&W DB-5ms UI, 15 m × 0.25 mm, 0.25 µm, GC columns.

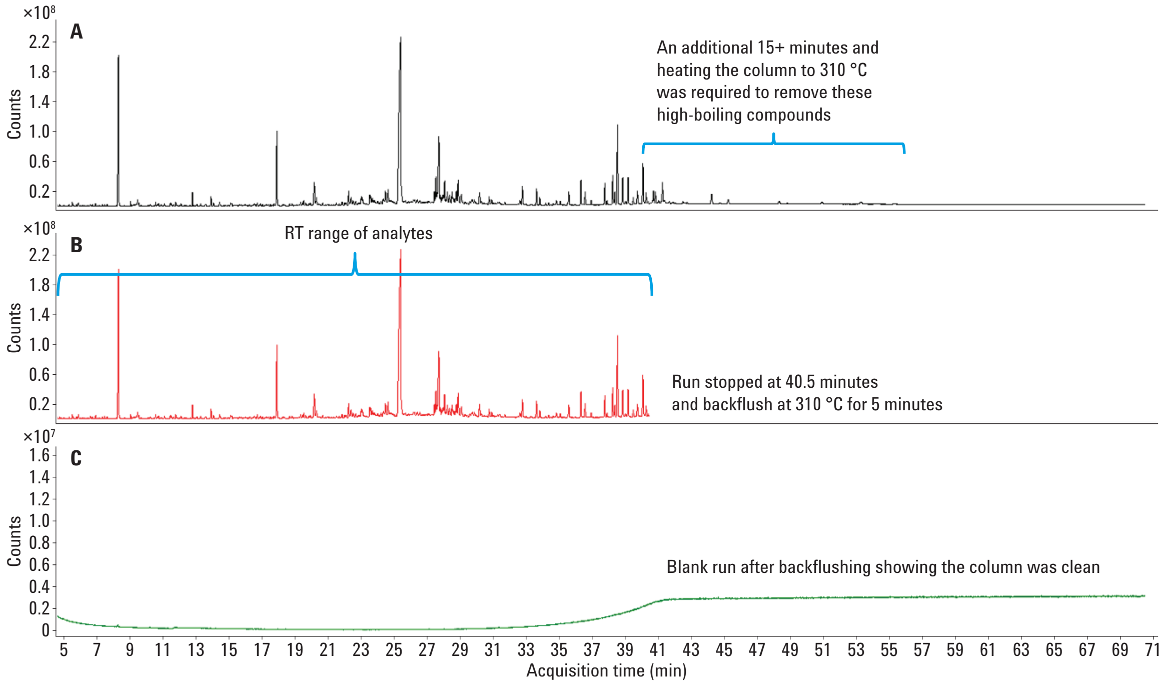

Figure 2. Backflushing cleaned the analytical columns and reduced the cycle time for target analytes in tobacco extracts.

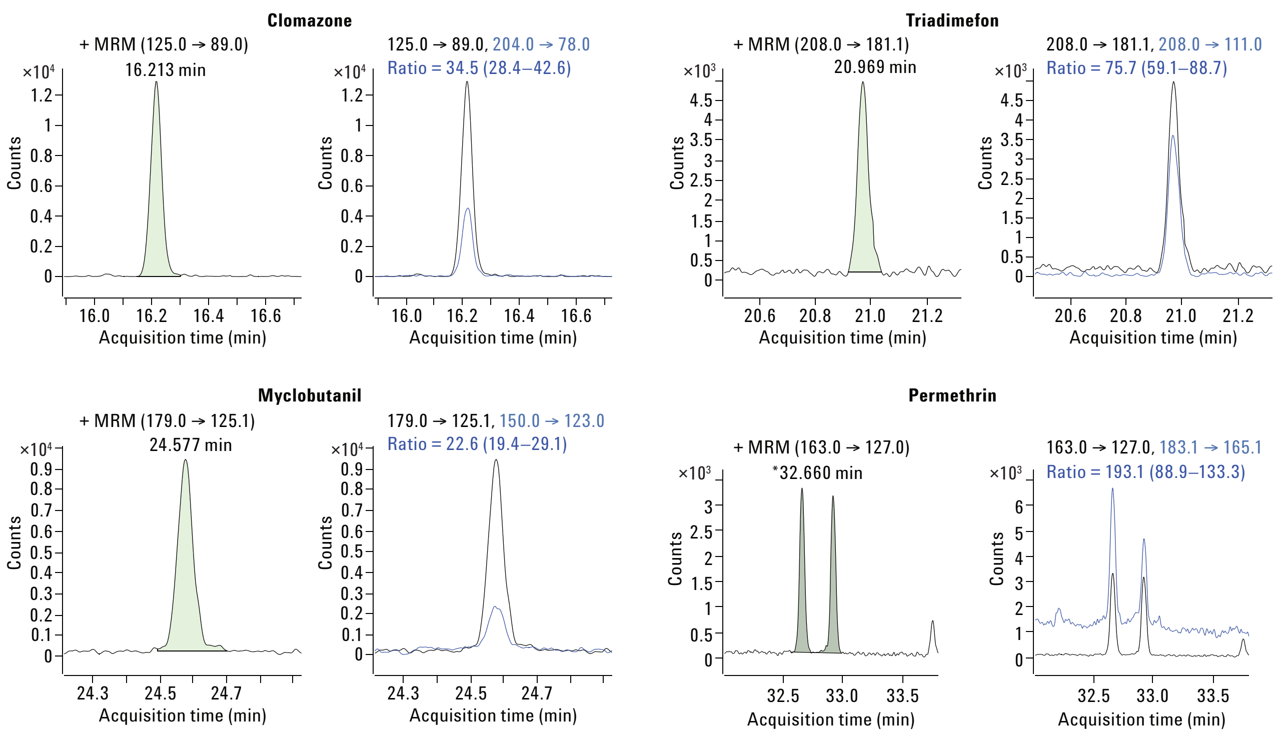

Figure 3. Chromatograms of clomazone, triadimefon, myclobutanil, and permethrin at 0.01 mg/kg after 200+ 1 µL injections of concentrated tobacco extracts.

Accurate calibration

A set of eight calibration matrix standards were injected consecutively, and yielded coefficient of correlation values (R^2) that were > 0.99 in over 92% of the cases. Naled, trifluralin, benfluralin, parathion-methyl, fenitrothion, isopropalin, pendimethalin, fensulfothion, chlornitrofen, fenamiphos-sulfoxide, fenvalerate, deltamethrin, and famoxadone yielded R^2 values from 0.98 to 0.99. Parathion and butralin yielded R^2 values from 0.97 to 0.98. Figure 4 shows calibration curves obtained in the tobacco matrix for six commonly detected pesticides in tobacco, namely triadimefon, metalaxyl, isoprothiolane, clomazone, acetamiprid, and cyfluthrin.

Some pesticides, especially organophosphates, degrade in solvents. For these pesticides, pure solid phase standards are suggested for storage. They need to be diluted and analyzed in a timely manner to achieve better linearity. The current MS gain factor setting in this study was 10. This may be lowered to achieve a larger linear range, if necessary.

LOQs well below MRLs

The LOD and LOQ calculations follow the EPA model, which is approached with replicates using a t-value at 99% confidence [11]. In this study, seven repetitive injections of tobacco matrix blanks with known low concentration levels of spiked pesticides were analyzed to calculate LOD and LOQ. LOQs of all 162 pesticides were well below the GRLs issued by ACAC [1].

Although all 162 pesticides can be analyzed with GC/MS, to achieve even lower LOQs, the preferred technique for some pesticides is LC/MS. These include some benzoylurea insecticides such as diflubenzuron, teflubenzuron, and triflumuron; some carbamates such as benfuracarb, carbaryl, carbofuran, 3-hydroxycarbofuran, carbosulfan, methiocarb, methomyl, oxamyl, and pirimicarb; more polar or otherwise problematic organophosphates such as acephate, azinphos-Et/Me, dimethoate, methamidophos, monocrotophos, naled, and omethoate; some neonicotinoids such as acetamipirid and thiamethoxam; and nonhalogenated pyrethroids.

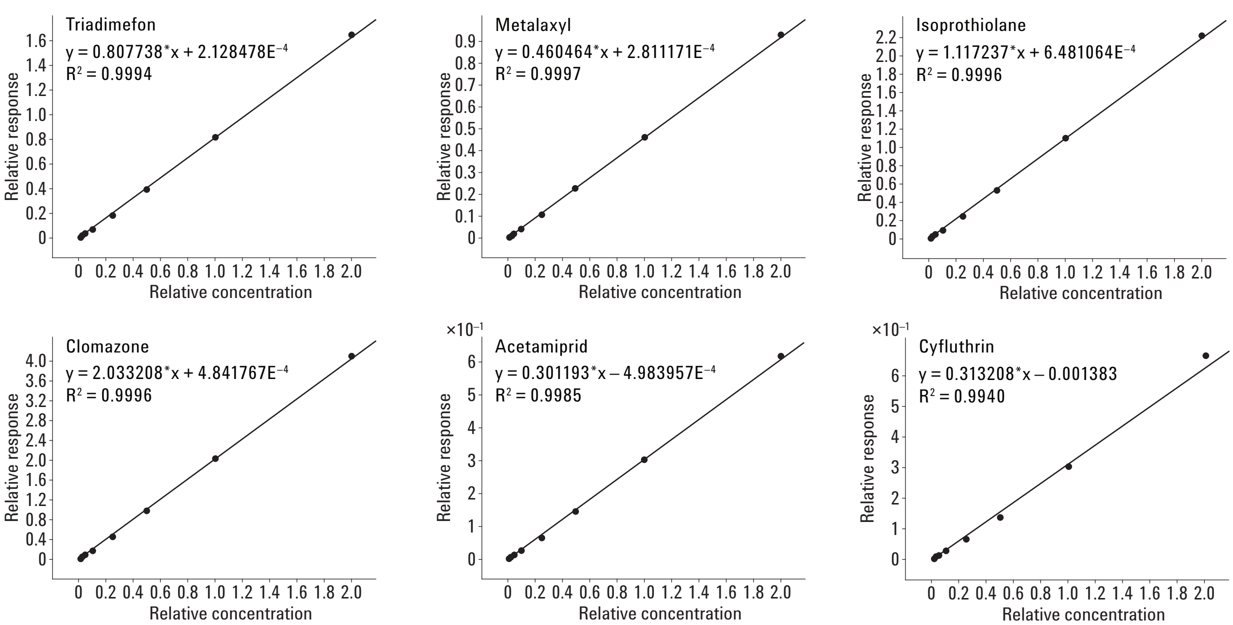

Figure 4. Calibration curves in a tobacco matrix for representative pesticides triadimefon, metalaxyl, isoprothiolane, clomazone, acetamiprid, and cyfluthrin.

Other features that can affect LOQs include pesticide stability and the lack of characteristic precursor ions using electron ionization. When using electron ionization, pesticides such as captafol and methoprene have fragmented EI spectra and lack characteristic precursors, product ions, or MRM transitions, that affect their LOQs. Degradation of pesticides is also a common reason why some pesticides do not have good LOQs. Because naled and trichlorfon degrade to dichlorvos even at room temperature, these three pesticides are sometimes analyzed together and quantified using dichlorvos, only. Teflubenzuron degrades to three main artifacts, and only the artifact with the highest response was analyzed in this study. Captan not only has a fragmented EI spectrum, but also tends to degrade to 1,2,3,6-tetrahydrophthalimide, which affect its LOD and LOQ.

Excellent RSDs

Figure 5A shows seven consecutive injections of each sample at concentration levels near LOQ and 0.1 mg/kg, respectively. Of 162 pesticides tested, 159 were at the concentration level near LOQ, and 161 at 0.1 mg/kg yielded %RSDs less than 15%.

Figure 5B shows the number of pesticides in tobacco with given %RSD values based on calculated amounts at two concentrations, 0.05 and 0.5 mg/kg. Percentage RSD values were obtained from three repetitive recovery study samples, and each was injected once on the GC/MS/MS. Of 162 pesticides tested, 160 at 0.05 mg/kg (98.8%), and 161 at 0.5 mg/kg (99.4%) yielded %RSD less than 15%.

In both studies, repeatability was excellent, showing less than 15% RSD for over 95% of the pesticides, even for the most challenging compounds omethoate, acephate, and DDT. Some pesticides, such as *p,p´*-DDT and methoxychlor, have similar structures and are known to degrade in the GC inlet, causing signal variability. In such cases, a suitable, compound-specific ISTD can be added to the final extract before instrumental analysis for signal normalization. For *p,p´*-DDT, the postextraction addition of a labeled ISTD ($^{13}C_{12}$-*p,p´*-DDT) can be a cost-effective way to address degradation and other potential GC-related issues.

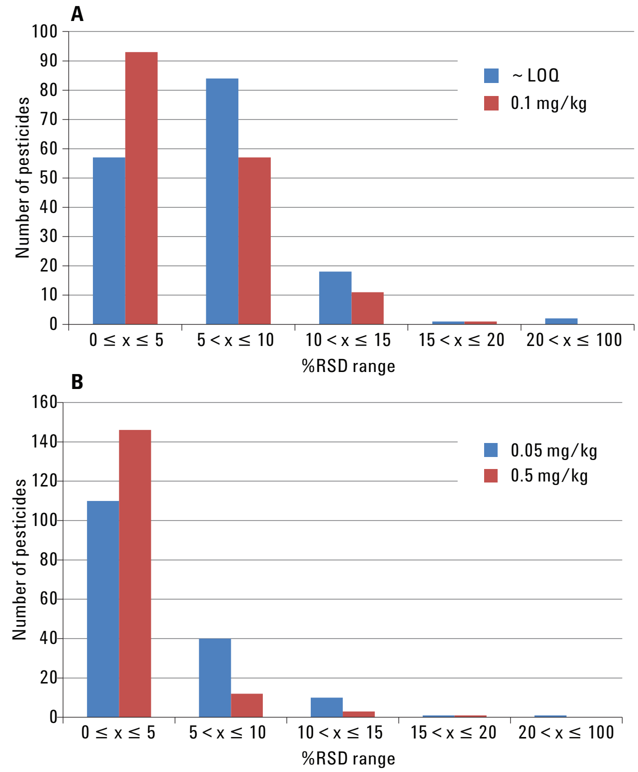

Figure 5. Distribution of %RSD of 162 pesticides in tobacco matrices: (A) seven consecutive injections of one sample at the concentration level near LOQ and at 0.1 mg/kg, (B) three repetitive recovery study samples at two different concentration levels of 0.05 and 0.5 mg/kg.

Matrix effects

Some pesticides showed consistent responses in different matrices, but some pesticides had different responses in different matrices due to either matrix enhancement or matrix suppression, and sometimes even slightly different retention time. Disulfoton sulfoxide, formothion, heptenophos, and tefluthrin spiked at 0.1 mg/kg were used as probes. Figure 6 shows the matrix effect of three different tobacco matrices from two different countries. The matrix enhancement of disulfoton sulfoxide and formothion in tobacco matrix 3 was stronger than the one in tobacco matrices 1 and 2. The retention time of disulfoton sulfoxide was also affected by different matrices. Heptenophos and tefluthrin showed consistent results in all three matrices. Therefore, matrix effect was compound dependent and it is important to use matrix-matched calibrations to achieve accurate quantitation results.

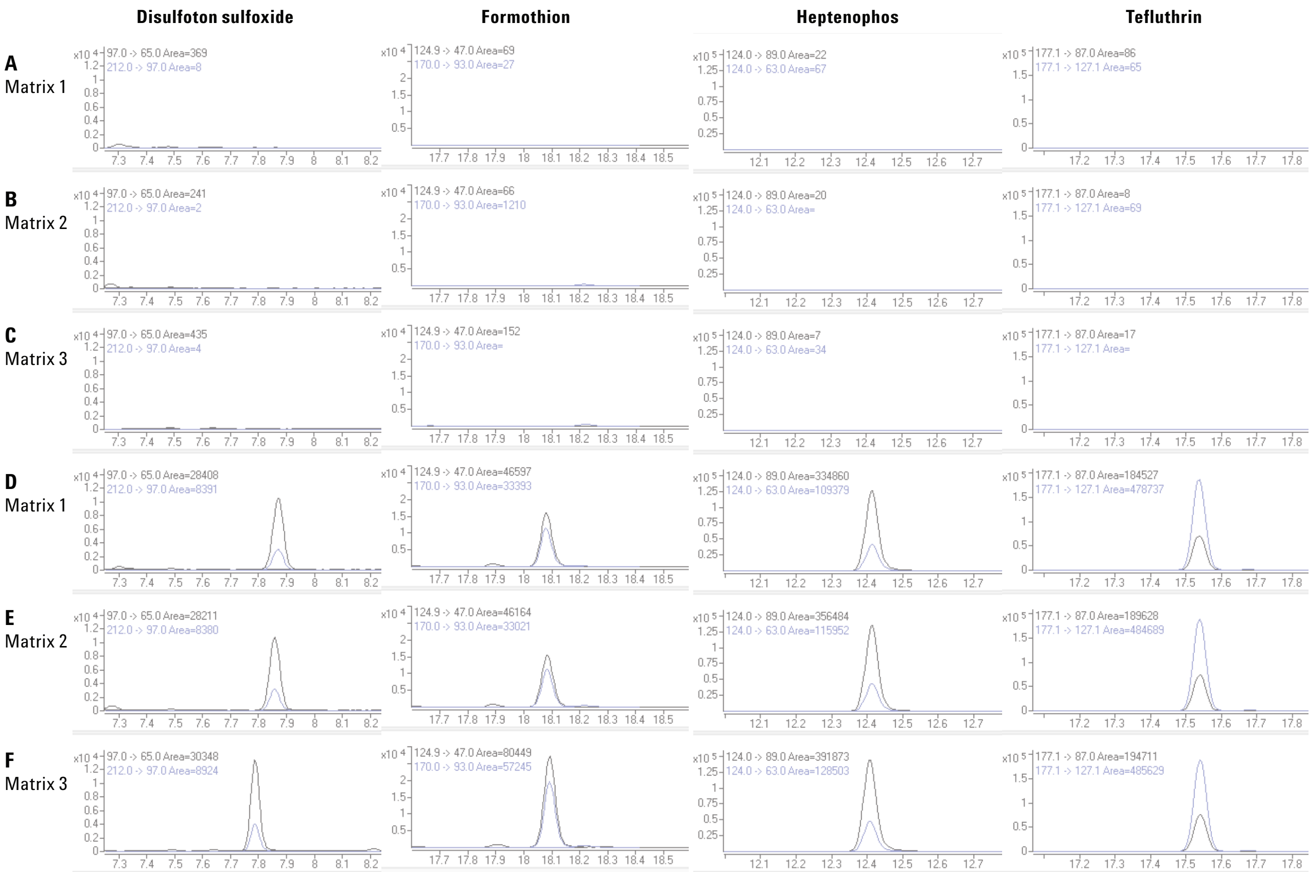

Figure 6. Chromatograms of disulfoton sulfoxide, formothion, heptenophos, and tefluthrin in three different tobacco matrices: (A-C) matrix blanks of three different tobacco matrices, (D-F) spiked at 0.1 mg/kg in three different matrix blanks.

Conclusions

The Agilent 7000C Triple Quadrupole GC/MS/MS Analyzer for Pesticides in Tobacco is a sensitive and rugged tool for target pesticide analysis in this complex matrix. The design of the system enables lower detection limits for pesticides when combined with an inert sample path and GC column backflushing. The high sensitivity EI Extractor Ion Source with improved thermal characteristics delivers confident trace analysis even in tobacco matrices, and the Triple-Axis HED-EM Detector reduces neutral noise by the doubly off-axis position of the HED-EM. These features enabled LOQs well below the GRLs issued by ACAC. Excellent linearity ($R^2 > 0.99$) for over 92% of 162 pesticides and excellent analysis repeatability (%RSD < 15%) for over 95% of 162 pesticides in tobacco matrices were achieved. The modified QuEChERS method using Agilent Bond Elut QuEChERS Extraction and Dispersive kits yielded excellent recoveries from 70 to 120% for 95% of all test pesticides.

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