Improved GC/MS Analysis of Tomato Pesticides with Agilent Deactivated Silica Tubing

Agilent Ultimate Plus Deactivated Fused Silica Tubing for Guard Columns

Application Note

Food Safety

Authors

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Abstract

Agilent Ultimate Plus deactivated fused silica tubing was evaluated as a guard column and compared with an equivalent and popular guard column from another vendor to analyze tomato samples extracted with QuEChERS. Ultimate Plus deactivated fused silica tubing can provide better linearity, repeatability, and stability. The Agilent deactivated FS tubing was superior to another vendor's tubing for the analysis of active and difficult pesticides in tomatoes.

Introduction

Guard columns are widely used in GC and GC/MS applications to protect the analytical column from contamination. When the guard column is a short piece of uncoated, deactivated fused silica tubing, the use of a guard column is also an inexpensive technique to extend the lifetime of capillary columns. Since contamination is limited to the front of the column, trimming the guard column periodically to restore performance, instead of the capillary column, preserves the main column. Thus, chromatography, including retention time and resolution, is not affected.

Due to complex matrices, multiresidue analysis of pesticides in fruits, vegetables, and other foods is always a challenge for sample preparation and detection. The QuEChERS method for pesticide analysis was introduced by USDA scientists in 2003 [1], and has been applied widely because of its "Quick, Easy, Cheap, Effective, Rugged and Safe" features. Agilent Bond Elut QuEChERS kits have demonstrated excellent recoveries for frequently used pesticides in tomato and other matrices [2,3]. However, food extracts processed by QuEChERS are still complicated, containing impurities such as high-boiling compounds that can cause contamination of the analytical column. Many pesticides are active analytes, and contamination of the analytical column leads to poor peak shape, loss of response, and shorter column lifetime. High inertness performance to minimize analyte degradation and peak tailing is required. To ensure accurate and reproducible results, using deactivated fused silica tubing as a guard column to protect the analytical column plays a key role in an inert flow path.

In this application note, tomato was selected because it is a high-consumption fruit in many cultures, but also because it is purported to have many health benefits derived from its lycopene content, which helps to decrease oxidative stress. Agilent Ultimate Plus deactivated fused silica tubing has shown excellent performance as a GC restrictor in the analysis of pesticide checkout mixtures [4], and as a guard column in an endrin/DDT breakdown test [5]. A representative group of difficult pesticides, including organophosphates (OPs), organochlorines (OCs), carbamates, and pyrethroids, were spiked in tomato matrix blank samples and extracted with Bond Elut QuEChERS kits. The matrix-spiked standards were then analyzed by GC/MS with an Agilent J&W HP-5ms Ultra Inert Column connected with Agilent Ultimate Plus deactivated fused silica tubing as a guard column.

In addition, tests were also performed on tubing from a different vendor for comparison under the same GC/MS conditions.

Experimental

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 Co. Ltd (Shanghai, China). Water was from J. T. Baker. Pesticide standards and the internal standard (triphenyl phosphate, TPP) were purchased from Ultra Scientific (North Kingstown, RI, USA) and J&K Scientific.

Matrix blank preparation

Organic tomatoes were bought from a local food market. The tomatoes were frozen, chopped, and then homogenized thoroughly. A 10.0 g (± 0.1 g) amount of homogenized sample was placed into a 50 mL centrifuge tube and prepared with QuEChERS, as shown in Figure 1. Extraction and cleanup were achieved using an Agilent Bond Elut QuEChERS EN extraction kit (p/n 5982-5650CH) and a Bond Elut QuEChERS dispersive kit (p/n 5982-0029).

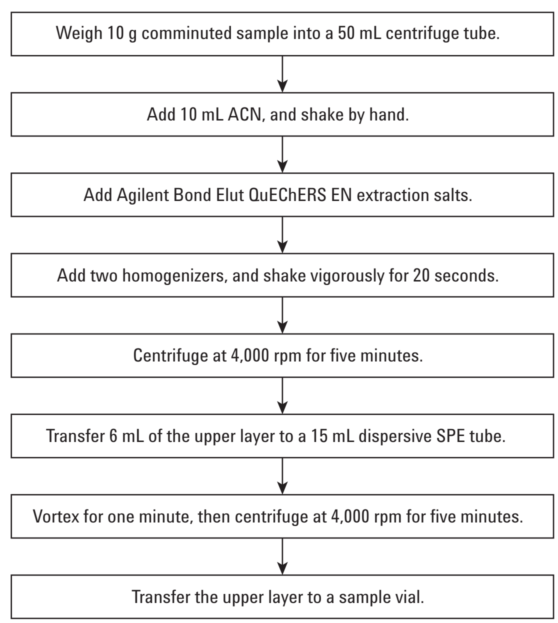

Figure 1. QuEChERS procedure to extract pesticide residues in tomatoes.

Solutions and standards

Standard and internal standard stock solutions (100 µg/mL) were made in acetonitrile and stored at –18 °C. Due to lower response of the pesticides in Group 1, including fipronil, chlorfenapyr, iprodione, cyfluthrin, fenvalerate, difenoconazole, deltamethrin, and azoxystrobin, the concentration of these compounds in pesticide working solutions was twice as high as in Group 2.

The high-QC solution, which was 20 µg/mL for Group 1 and 10 µg/mL for Group 2 pesticides, was prepared in toluene. This solution was used to prepare calibration curves in the matrix blank extract by appropriate dilution. Internal standard solution was added to give a final concentration of 100 ng/mL.

Instrumentation

Instruments and conditions are shown in Table 1. Two guard columns with different serial numbers were purchased from each supplier to demonstrate performance of different manufacturing lots. Two guard columns from each serial number were tested to confirm reproducibility. All guard columns were connected to analytical columns using Agilent Ultimate Unions, and analyzed in the same manner. Table 2 lists the flow path consumable supplies.

Table 1. Instrumental conditions.

Analytical column	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI) Agilent Ultimate Plus deactivated FS tubing, 5 m × 0.25 mm (p/n CP802505)
Guard column	Deactivated FS tubing, 5 m × 0.25 mm, from supplier R
GC	Agilent 7890B GC
Autosampler	Agilent 7683B Autosampler and sample tray, 5 µL syringe (p/n G4513-80213), 2 µL injection volume
Carrier gas	Helium, constant flow mode Split/splitless, 250 °C, pulsed splitless, 25 psi pulse pressure for 0.75 min
Inlet	50 mL/min purge flow at 0.75 min
RT locking	Chlorpyrifos-methyl locked to 13.443 min
Oven	50 °C (1 min), 25 °C/min to 125 °C (0 min), 10 °C/min to 300 °C (10 min)
MSD	Agilent 5977A MSD
Solvent delay	4 min
MS temperature	300 °C (source), 150 °C (quad)
Transfer line	280 °C
MS	EI, SIM
Other parameters	see Table 3

Table 2. Flow path supplies.

Vials	Amber, write-on spot, certified, 2 mL, screw top vial packs (p/n 5182-0554)
Vial Inserts	150 µL glass with polymer feet (p/n 5183-2088)
Septa	Nonstick BTO septa (p/n 5183-4757)
Column nut	Self-tightening, inlet/detector (p/n 5190-6194)
Ferrules	15% graphite: 85% Vespel, short, 0.4 mm id, for 0.1 to 0.25 mm columns (10/pk, p/n 5181-3323) UltiMetal Plus Flexible Metal, 0.4 mm id, for 0.1 to 0.25 mm fused silica tubing (10/pk, p/n G3188-27501)
Union	Agilent Inert Ultimate union (p/n G3182-60581)
Liner	Agilent Ultra Inert deactivated single taper splitless liner with wool (p/n 5190-2293)
Inlet seal	Ultra Inert, gold-plated, with washer (p/n 5190-6144)
Internal nut	CFT capillary fitting (p/n G2855-20530)

Table 3. Pesticides, CAS number, target ion, and retention time.

No.	**Compound**	**CAS no.**	**Target ion**	**RT**	**No.**	**Compound**	**CAS no.**	**Target ion**	**RT**
ISTD	Triphenyl phosphate	115-86-6	326	17.997	35	Parathion	56-38-2	291	14.401
1	Methamidophos	10265-92-6	94	6.412	36	Triadimefon	43121-43-3	57	14.439
2	DDV	62-73-7	109	6.619	37	Dicofol	115-32-2	139	14.441
3	Mevinphos	298-01-1	127	8.474	38	Isocarbophos	24353-61-5	136	14.519
4	Acephate	30560-19-1	136	8.481	39	Bromophos-methyl	2104-96-3	331	14.738
5	Heptenophos	23560-59-0	124	10.119	40	Isofenphos-methyl	99675-03-3	199	14.889
6	Omethoate	1113-02-6	156	10.277	41	Pendimethalin	40487-42-1	252	15.008
7	Propoxur	114-26-1	110	10.477	42	Fipronil	120068-37-3	367	15.165
8	Ethoprophos	13194-48-4	158	10.729	43	Quinalphos	13593-03-8	146	15.232
9	Cadusafos	95465-99-9	159	11.300	44	Methidathion	950-37-8	145	15.511
10	Phorate	298-02-2	75	11.391	45	Tetrachlorvinphos	22248-79-9	329	15.685
11	*alpha*-BHC	319-84-6	181	11.511	46	Fenamiphos	22224-92-6	303	15.869
12	Dimethoate	60-51-5	87	11.749	47	Profenofos	41198-08-7	208	16.095
13	Carbofuran	1563-66-2	164	11.872	48	Chlorfenapyr	122453-73-0	59	16.633
14	Atrazine	1912-24-9	200	11.930	49	4,4'-DDD	72-54-8	235	16.951
15	*beta*-BHC	319-85-7	219	12.041	50	Ethion	563-12-2	231	17.030
16	*gamma*-BHC	58-89-9	181	12.178	51	Triazophos	24017-47-8	161	17.250
17	Quintozene	82-68-8	237	12.281	52	4,4'-DDT	50-29-3	235	17.638
18	Fonofos	944-22-9	109	12.339	53	Iprodione	36734-19-7	187	18.326
19	Diazinon	333-41-5	179	12.480	54	Phosmet	732-11-6	160	18.518
20	Disulfoton	298-04-4	88	12.591	55	Phosalone	2310-17-0	182	19.212
21	Tefluthrin	79538-32-2	177	12.677	56	*lambda*-Cyhalothrin	91465-08-6	181	19.527
22	Chlorothalonil	1897-45-6	266	12.744	57	Cyfluthrin I	68359-37-5	163	20.825
23	Pirimicarb	23103-98-2	166	12.999	58	Cyfluthrin II	68359-37-5	163	20.914
24	Phosphamidon	13171-21-6	127	13.243	59	Cyfluthrin III and IV	68359-37-5	163	21.003
25	Vinclozolin	50471-44-8	212	13.422	60	Cypermethrin	52315-07-8	181	21.145
26	Methyl parathion	298-00-0	263	13.435	61	Cypermethrin	52315-07-8	181	21.228
27	Chlorpyrifos-methyl	5598-13-0	286	13.444	62	Cypermethrin	52315-07-8	181	21.331
28	Carbaryl	63-25-2	144	13.530	63	Fenvalerate	51630-58-1	167	22.070
29	Fenchlorphos	299-84-3	285	13.728	64	Fenvalerate	51630-58-1	167	22.236
30	Demeton-S-methyl sulfone	17040-19-6	169	13.799	65	Difenoconazole	119446-68-3	323	22.529
31	Fenitrothion	122-14-5	277	13.973	66	Difenoconazole	119446-68-3	323	22.614
32	Malathion	121-75-5	173	14.161	67	Indoxacarb	173584-44-6	150	22.814
33	Fenthion	55-38-9	278	14.339	68	Deltamethrin	52918-63-5	181	22.932
34	Chlorpyrifos	2921-88-2	197	14.385	69	Azoxystrobin	131860-33-8	344	23.256

Results and Discussion

The purpose of the tests was to evaluate the performance of Agilent Ultimate Plus deactivated FS tubing as a guard column, and compare it with a popular deactivated guard column from another supplier for the analysis of pesticides in a vegetable matrix by GC/MS. The system was inspected and carefully cleaned, if necessary, before each test. For consistency, new UI columns, UI gold seals, liners, and inert unions were used for each tubing test.

Protecting the analytical column

One potential issue with the use of GC/MS for analysis of QuEChERS samples is contamination and deterioration of the GC column. QuEChERS vegetable samples usually still contain some impurities that can accumulate on the head of the column, causing peak tailing, retention time shifting, and reduced response. Figure 2 shows chromatograms of a blank tomato extract and a 50 ng/mL spiked QuEChERS sample analyzed with deactivated FS tubing from different suppliers. As shown in Figure 2C, interference peaks are found in the blank chromatogram. These interference peaks are either completely separated with the target pesticide's peak, or with significantly lower intensity compared to the target pesticide's peak. Therefore, these interference peaks do not affect integration and quantitation of the pesticides of interest. However, these impurities can also cause some chromatographic problems in that peak shape and intensity may deteriorate faster as more complicated samples are injected.

Using Agilent Ultimate Plus deactivated FS tubing as a guard column is an effective way to protect the analytical column by trimming the tubing periodically instead of the analytical column, to restore performance.

Backflushing is recommended for complicated sample matrices to reduce analysis time, frequency of MSD source cleaning, and column head trimming [6]. Backflushing was not used in this study to reduce influences on the test results. However, we recommend the use of Ultimate Plus deactivated FS tubing as a guard column and backflushing in routine pesticide analysis.

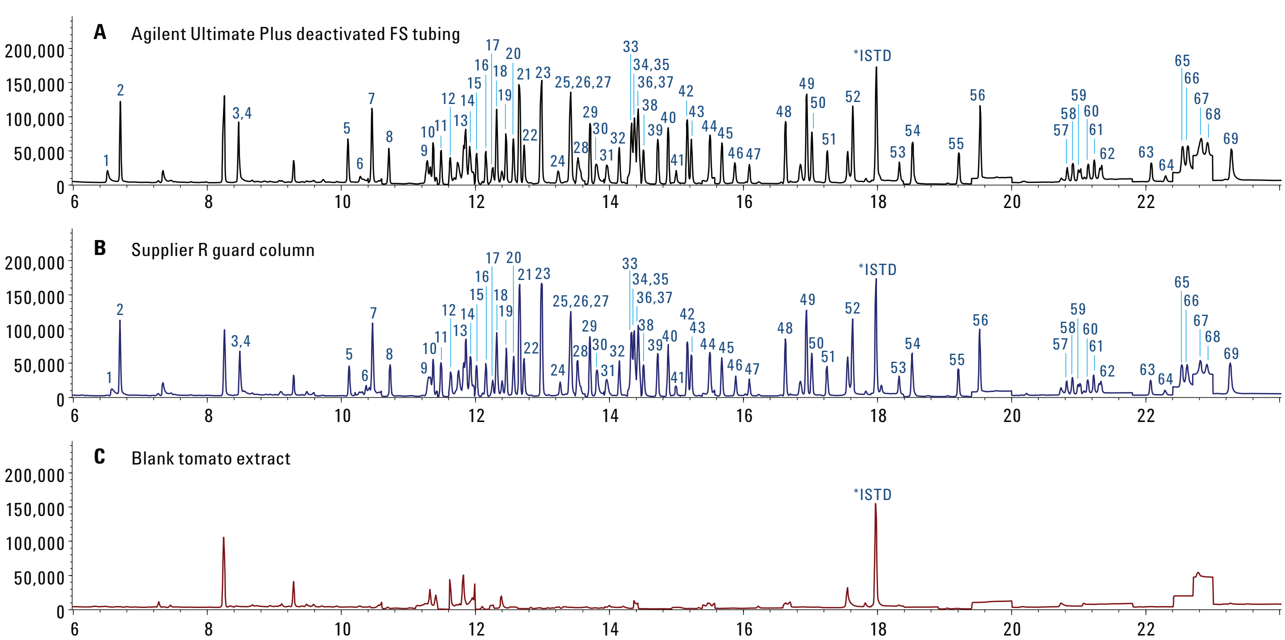

Figure 2. GC/MS chromatogram of tomato extract using deactivated FS tubing as guard columns. A) Agilent Ultimate Plus deactivated FS tubing, B) supplier R guard column, and C) tomato extract blank (peak identification in Table 3).

Important components in the flow path

Flow path inertness plays a critical role in the accuracy, precision, durability, and consistency of pesticide analysis in complicated sample matrices. Each component of the flow path has the potential to contribute to overall system activity. Therefore, the Agilent Inert Flow Path solution, which provides excellent surface inertness for the entire GC flow, includes Ultra Inert columns, Ultra Inert inlet liners and gold seals, with UltiMetal Plus inert inlet, capillary flow technology (CFT) devices, and other inertness-verified consumables. As shown in Table 4, the surface areas of liners and gold seals are about 4.4 and 1 cm^2, respectively; while the surface area of a 5 m × 0.25 mm guard column is about 39.3 cm^2. If surface area is proportional to the potential for active sites, this means that the guard column has over eight times more active sites than the liner, and 39 times more active sites than the gold seal in the system. Chromatographically active compounds such as organophosphate pesticides can adsorb onto active sites, particularly at trace levels, compromising an analyte's response. High inertness performance of deactivated fused silica tubing is very important for the entire GC flow path.

Table 4. GC Flow path surface areas

	Length (cm)	Diameter (cm)	Surface area (cm^2)
Liner	7	0.2	4.4
Seal	0.4	0.8	1
Column	3,000	0.025	235.6
Guard column	500	0.025	39.3

Performance comparison

Pesticides of interest in this study were from various pesticides groups, such as organophosphates, organochlorines, carbamates, and pyrethroids. These compounds also included many difficult active pesticides such as methamidophos, acephate, omethoate, dimethoate, carbaryl, chlorothalonil, DDT, phosmet, and iprodione. Evaluation and comparison focused on the performance of the guard column for these active compounds and some pesticides for routine inspection.

Due to the inert flow path, most analytes showed sharp and symmetrical peak shapes with Ultimate Plus deactivated FS tubing and supplier R guard columns (see Figure 2A and Figure 2B).

The more polar pesticides can be problematic, often yielding broad peak shapes or excessive tailing that makes reliable quantitation at low levels difficult. Figure 3 depicts a GC/MS/SIM chromatogram of more problematic polar pesticides on different supplier's tubing. Compared to supplier R's guard column, the high level of inertness of Ultimate Plus deactivated FS tubing results in better peak shape and decreased sample adsorption, allowing lower detection limits.

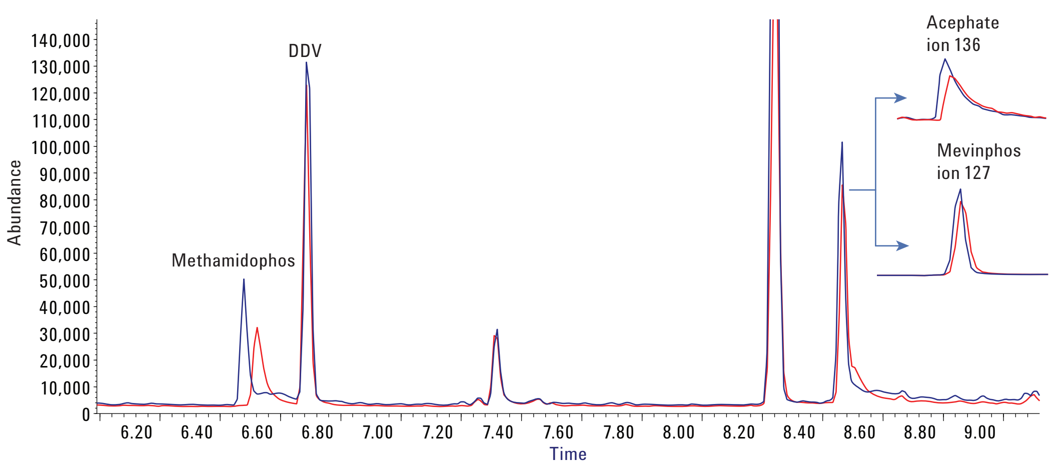

Figure 3. Enlarged section of GC/MS chromatogram of more problematic polar pesticides. Agilent Ultimate Plus deactivated FS tubing (blue), and supplier R guard column (red).

Linearity

Linearity was determined using calibration curves spiked into the tomato matrix. Calibration curves were constructed from 2 µL injections of selected standards at 10, 20, 50, 100, 250, and 500 ng/mL. Every standard solution contained 100 ng/mL of internal standards. Figure 4 compares calibration curve coefficients (R^2) for Ultimate Plus deactivated FS tubing and the supplier R guard column. The supplier R guard column is widely recognized for its inertness performance. All pesticides showed excellent linearity with calibration coefficients greater than 0.990 on both Ultimate Plus deactivated FS tubing and the guard column from supplier R. Overall, however, Agilent deactivated FS tubing exhibited better performance, particularly for very difficult pesticides, such as methamidophos, acephate, and omethoate.

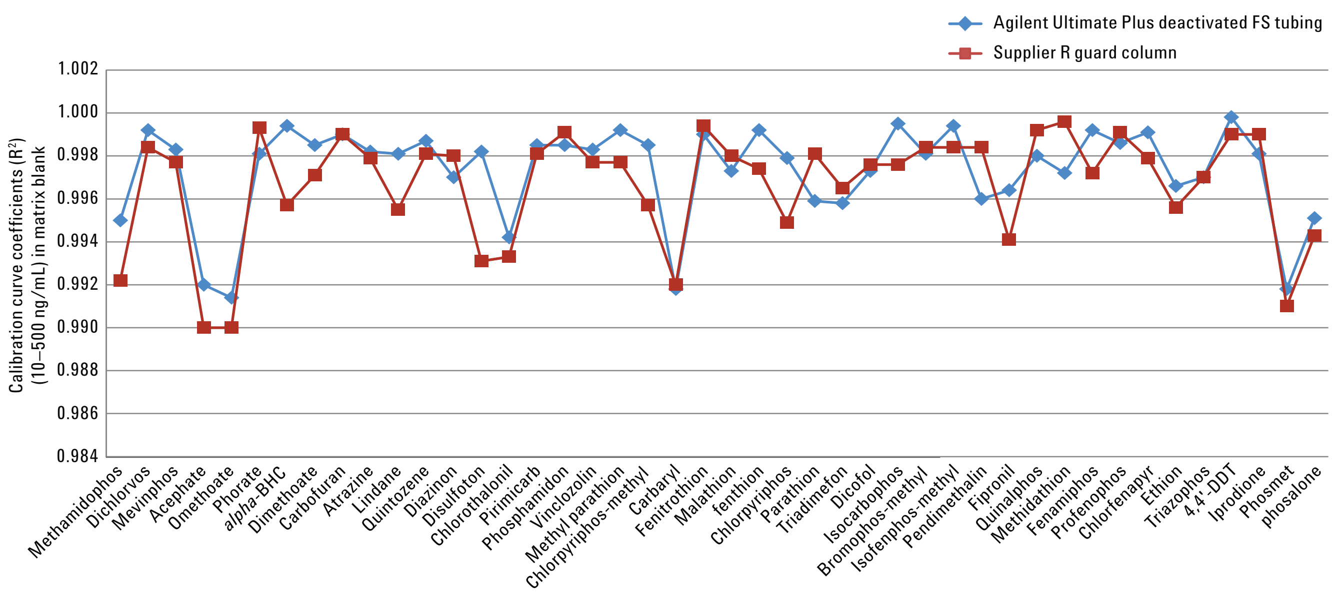

Figure 4. Comparison of calibration curve coefficients (R^2) for Agilent Ultimate Plus deactivated FS tubing and supplier R guard column.

Repeatability and stability

A 50 ng/mL spiked QuEChERS sample was analyzed over 15 injections to test repeatability and stability. The peak-area ratios of analytes/internal standards for most analytes were comparable between Ultimate Plus deactivated FS tubing and the supplier R guard column. However, for difficult active pesticides, better RSD values meant that the Agilent tubing provided more consistent responses of active pesticides, and thus supported more sample runs with acceptable results (Table 5).

Conclusions

Agilent Ultimate Plus deactivated fused silica tubing was evaluated for use as a guard column in the analysis of tomato QuEChERS samples, and compared with tubing from another supplier. Convincing proof for the surface inertness improvement of Ultimate Plus deactivated fused silica over another vendor's deactivated tubing includes better calibration curve linearity and longer durability, demonstrated by slower and reduced signal drop over multiple injections of critical active pesticides. This was visually apparent, and verified by RSD values. Ultimate Plus deactivated fused silica tubing can be used to improve GC flow path performance when used as guard columns for pesticide analysis in complicated matrices.

Table 5. Injection repeatability and performance stability.

	Injection repeatability (RSD%) n=15		
No.	**Pesticide**	**Supplier R guard column**	**Agilent Ultimate Plus deactivated FS tubing**
1	Methamidophos	6.69	5.86
2	Dichlorvos	2.25	2.14
3	Acephate	10.81	8.87
6	Omethoate	10.74	6.51
10	Phorate	1.36	1.15
12	Dimethoate	3.38	3.41
13	Carbofuran	3.73	3.15
14	Atrazine	1.18	1.26
16	Lindane	1.61	0.96
17	Quintozene	2.91	3.02
19	Diazinon	1.65	0.90
20	Disulfoton	1.59	1.66
22	Chlorothalonil	5.10	3.42
23	Pirimicarb	3.20	1.51
24	Phosphamidon	1.96	2.22
25	Vinclozolin	4.04	2.50
26	Methyl parathion	4.23	1.29
28	Carbaryl	7.42	6.17
31	Fenitrothion	2.28	1.67
32	Malathion	1.51	1.43
34	Chlorpyrifos	5.00	4.79
35	Parathion	5.04	2.78
36	Triadimefon	1.32	2.73
37	Dicofol	6.31	2.10
38	Isocarbophos	1.55	1.71
40	Isofenphos-methyl	1.37	1.34
41	Pendimethalin	3.48	1.52
42	Fipronil	3.48	1.89
43	Quinalphos	0.70	1.04
47	Profenofos	3.79	3.73
50	Ethion	2.83	1.24
51	Triazophos	2.28	1.21
52	4,4'-DDT	1.25	0.65
53	Iprodione	2.88	2.46
54	Phosmet	3.37	1.98
55	Phosalone	3.98	2.36

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