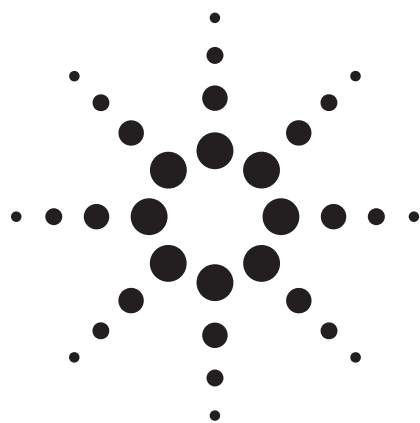


A Direct Column-Performance Comparison for Rapid Contract Laboratory Program (CLP) Pesticide Analysis



Application

Environmental

Authors

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Abstract

Agilent J&W High Efficiency GC columns with internal diameter of 0.18 mm for Contract Laboratory Program (CPL) pesticide analyses gave superior results for CPL pesticide primary analysis and confirmation. Chromatograms depicting peak shape characteristics, peak resolution, and baseline stability for two sets of 0.18-mm id columns are presented in a head-to-head comparison. Complete primary and confirmatory analysis of the 20 pesticides in the protocol is accomplished in less than 6 minutes using hydrogen carrier gas and flow programming. Successful primary and confirmatory analyses were achievable only on Agilent J&W High Efficiency GC columns.

Introduction

The analyses of organochlorine pesticides (OCPs) in environmental remediation samples are important, high volume, analyses in the competitive contract laboratory marketplace. A standard Contract Laboratory Program (CLP) pesticide method is used for these analyses. In many cases a lab will analyze large numbers of samples over the course of a given project, accumulating costs to both the lab and its client. Use of Agilent J&W 0.18-mm id High Efficiency GC columns is a means of enhancing laboratory productivity [1-3].

Wool and Decker [3] reported their findings at the U.S. Environmental Protection Agency (Region VI, Houston, TX) laboratory and described the value of columns in the 20 m × 0.18 mm format for CLP pesticide analysis. Their suggestion to use a retention gap to protect the analytical columns from deleterious matrix effects and to help offset the lower sample capacity of these columns relative to wider bore columns was incorporated into this column comparison. Deactivated 5 m × 0.25 mm id retention gaps were used in this series of experiments on each column set used.

Columns with 0.18 mm id capable of doing CPL pesticide analysis are available from several leading column manufacturers. Agilent's suggested pair for CLP pesticide analysis in the 0.18 mm id format is a DB-17ms column for primary analysis and a DB-XLB column for confirmation. Vendor R's offering is a set of proprietary phase 0.18 mm id columns for both primary and confirmation analysis of CLP pesticides.

Experimental

The chromatograph used was an Agilent 6890N GC equipped with dual electron capture detectors (μ ECDs) and a 7683B autosampler. Sample introduction was done by a single split/splitless injection port at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the initial chromatographic conditions appear in Table 1.



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Table 1. Chromatographic Conditions

GC :	Agilent 6890N
Sampler:	Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Carrier:	Hydrogen (flow programmed, 69 cm/s at 120 °C, ramped at 99 mL/min to 106 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RSMH-2)
Inlet:	Split/splitless; 220 °C, pulsed splitless (35 psi for 0.5 min, purge flow of 40 mL/min on at 1 minute, gas saver flow 20 mL/min on 3 minutes
Inlet liner:	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:	5 m × 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:	Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) 2 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222)
Oven:	120 °C (0.32 min); 120 °C/min to 160 °C; 30 °C/min to 258 °C (0.18 min); 38.81 °C/min to 300 °C (1.5 min)
Detection:	µECD 320 °C; nitrogen makeup; constant column + makeup flow (60 mL/min)

The flow path supplies used in these experiments are listed in Table 2.

Table 2. Flow Path Supplies

	Description	Agilent p/n
Vials:	Amber screw cap	5182-0716
Vial caps:	Blue screw cap	5282-0723
Vial inserts:	100 µL glass/polymer feet	5181-1270
Syringe:	5 µL	5181-1273
Septum:	Advanced green	5183-4759
Inlet liner:	Deactivated single taper direct connect	G1544-80730
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite	5181-3323
Y-splitter:	Quartz deactivated	5181-3398
Sealing resin:	Polyimide sealing resin	500-1200
20x magnifier:	20x magnifier loupe	430-1020
Tubing cutter:	Ceramic wafer column cutter	5181-8836

Both sets of columns used in this comparison were installed into the GC in the same manner. The same retention gap and inlet liner were used for both sets of columns. The chromatographic conditions (except for the columns) in Tables 1 and 2 were used to evaluate both the proprietary columns recommended by Vendor R and Agilent's columns. The primary analysis column from Agilent was a 20 m × 0.18 mm × 0.18 µm DB-17ms. The column from Vendor R was a 20 m × 0.18 mm × 0.18 µm with a proprietary stationary phase. The confirmatory analysis column from Agilent was a 20 m × 0.18 mm × 0.18 µm DB-XLB. The column from Vendor R was a 20 m × 0.18 mm × 0.14 µm with a proprietary stationary phase.

Sample Preparation

CLP pesticide standard solutions were purchased from AccuStandard (New Haven, CT 06513 USA). ULTRA RESI ANALYZED grade 2,2,4 trimethylpentane was purchased from J.T. Baker (Phillipsburg, NJ 08865 USA).

CLP-023R-160X and CLP-024R-160X concentrates were diluted first into 50-mL volumetric flasks in 2,2,4 trimethylpentane and then serially diluted. Volumetric flasks and pipettes used were all class A. The standard concentration range for low-level target compounds in the protocol was from 3.2 to 80 ng/mL. On-column loading ranged from 0.8 to 20 pg for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Using Y Splitters

Installation of the Y splitter was accomplished by coating the outside of the fused silica tubing to be inserted into the Y splitter with a thin film of polyimide sealing resin prior to cutting the tubing. The cut was then made through the coated section of tubing. The cut end was then checked with a 20x magnification loupe to make sure that the cut was clean and that excess sealant had not diffused inside the column. Once a clean cut was obtained, the fused silica with the polyimide sealant on the outside only was inserted into the desired branch of the Y and held for approximately 45 seconds to seal. Good sealing was indicated by a thin ring of sealant at the point of contact. The process was done first with the analytical columns and then repeated for the trunk of the Y into the retention gap. This approach has given tight, reliable connections that have lasted without difficulty for over 2 months and through hundreds of oven temperature program cycles.

Method Translation Software/Path to Successful Conditions

The starting point for this comparison was the conversion of a successful set of separation conditions using helium carrier on Agilent's DB-17ms and DB-XLB 0.18 mm id columns [4] to a set of conditions using hydrogen carrier. The chromatographic parameters for the helium carrier separation were keyed into the translation table in the Agilent GC Method Translation software [5–6] to convert the method to use with hydrogen carrier. In the software, the "Translate Only" mode was used to convert the 11-minute helium carrier method to a 7.3-minute hydrogen carrier method using the same columns.

Method development effort beyond conversion from helium carrier to hydrogen carrier gas became necessary only when the goal of the analysis shifted to emphasize speed of analysis using flow programming. Flow programming is outside the scope of the Method Translation software. In this series of experiments, flow programming helped to elute highly retained peaks faster. Further temperature program modifications also increased the speed of analysis with minimal loss of resolution on the Agilent columns.

Results and Discussion

Successful separation of CLP pesticides using hydrogen carrier was demonstrated on Agilent's DB-17ms and DB-XLB 0.18-mm id columns using the conditions shown in Table 1. Vendor R's 0.18-mm ID columns were evaluated using the following conditions: the conditions shown in Table 1, the conditions obtained on Vendor R's Web site (to the extent practical), and with a set of conditions optimized specifically on Vendor R's columns for this analysis. The goal throughout these experiments was to show as fair and objective a comparison as possible.

To compare chromatograms, injections at a standard concentration of 3.2 ng/mL for low-level target species in the CLP protocol were selected. Using this concentration consistently provides a fixed point of reference and at the same time alleviates the potential for masking deleterious chromatographic effects often seen at higher concentrations. Inclusion of a Y scale in each chromatogram provides another fixed reference within each figure to facilitate comparison. Key aspects to

look for in the example chromatograms are peak resolution, indications of peak tailing, and temperature-dependent drift on the μ ECD.

An Agilent DB-17ms column was used as the primary analysis column in these experiments. An example chromatogram from an injection at a nominal concentration of 3.2 ng/mL for low target level pesticides is shown in the upper portion of Figure 1. This column resolved all the peaks of interest in less than 6 minutes, gave sharp symmetrical peaks, and had minimal background drift on the μ ECD. A compound label key for the numbered peaks in the chromatogram is located in Table 3.

An Agilent DB-XLB 20 m x 0.18 mm x 0.18 μ m column was used for confirmatory analysis on these experiments. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in the lower portion of Figure 1. This column resolved 20 of the peaks of interest in less than 6 minutes and gave near baseline resolution of peaks 10 and 11. Again, sharp symmetrical peaks and minimal temperature-dependent baseline drift were observed on the μ ECD. Although complete resolution of 20 of the 22 peaks of interest on the confirmatory columns is not ideal, the observed resolution is satisfactory for peak confirmation.

The peak identification table applies to Figure 1, depicting CPL pesticide separation on Agilent's column only. Elution order for these columns with their particular selectivity was established in previous work. To establish elution order on Vendor R's columns with different selectivity, injection of individual standards or mass spectral confirmation is required.

Table 3. CLP Standard Compound List Key

1. Tetrachloro-m-xylene	12. 4,4' DDE
2. Alpha BHC	13. Dieldrin
3. Gamma BHC	14. Endrin
4. Beta BHC	15. 4,4' DDD
5. Delta BHC	16. Endosulfan II
6. Heptachlor	17. 4,4' DDT
7. Aldrin	18. Endrin aldehyde
8. Heptachlor epoxide	19. Endosulfan sulfate
9. Gamma chlordane	20. Methoxychlor
10. Alpha chlordane	21. Endrin ketone
11. Endosulfan I	22. Decachlorobiphenyl

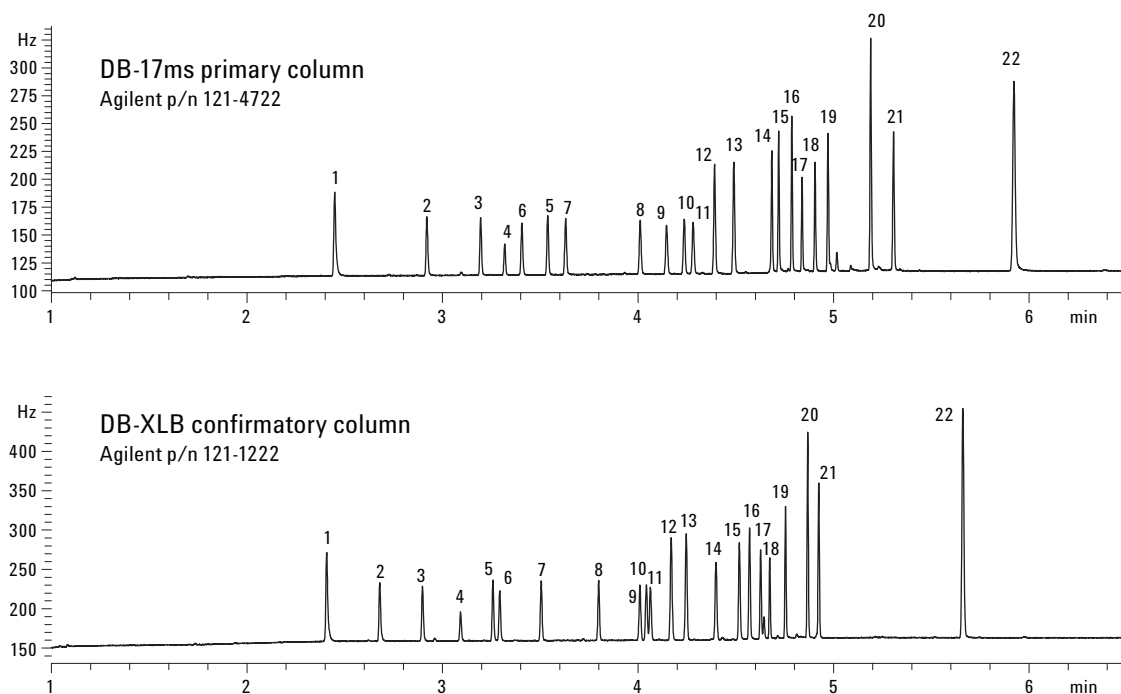


Figure 1. Chromatogram of 0.5- μ l injection of 3.2 ng/ml low-level target pesticide standard solution injected through a Y-splitter onto DB-17ms (Agilent p/n 121-4722) primary analysis column and a DB-XLB (Agilent p/n 121-1222) confirmatory analysis column, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m \times 0.18 mm \times 0.18 μ m with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column gave resolution of 20 of the 22 peaks of interest, peak tailing for some species, and minimal temperature dependent baseline drift on the μ ECD. The arrows within the figure point to co-eluting and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m \times 0.18 mm \times 0.14 μ m with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 2. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the μ ECD. The arrows within the figure point to tailing peaks and highlight baseline drift, in this case over 100 Hz.

Vendor R's suggested separation conditions for their column pair were unsuccessful at producing results equivalent to those shown on their Web site. This appears to stem from an oversight on their part. Suggested conditions found in a figure caption on the Web site called for a 2-min hold 10 $^{\circ}$ C above the maximum recommended temperature. A temperature of 330 $^{\circ}$ C was called for; however, the label on the column box listed the upper temperature program limit as 320 $^{\circ}$ C for the confirmation column. Vendor R's confirmation column demonstrated significant bleed even with a temperature program that reached only 300 $^{\circ}$ C, a full 20 $^{\circ}$ C below the upper limit.

A series of attempts to resolve the co-eluting pair of pesticides on Vendor R's primary analysis column gave improved but still incomplete resolution. It was necessary to substantially reduce flow rate and modify both temperature and flow programming parameters to achieve the results shown in Figure 3. The chromatographic conditions used for these injections appear in Table 4; the flow path supplies were the same as those listed in Table 2.

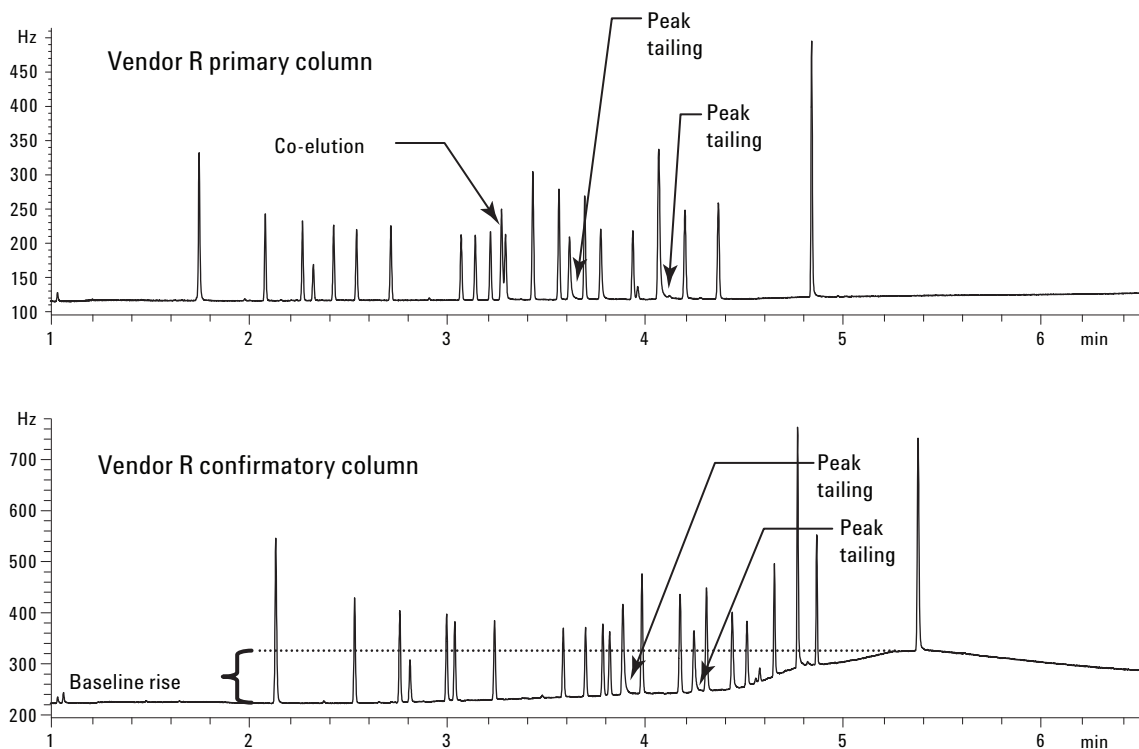


Figure 2. Chromatogram of 0.5 μ L injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1. Figure 2. Chromatogram of 0.5 μ L injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary analysis column and confirmatory columns, conditions as in Table 1.

The primary analysis column from Vendor R was a 20 m \times 0.18 mm \times 0.18 μ m with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column still gave resolution of 20 of the 22 peaks of interest, indication of peak tailing for some species, and minimal temperature-dependent baseline drift on the μ ECD. The arrows within the figure point to the unresolved peaks and tailing peaks.

The confirmatory analysis column from Vendor R was a 20 m \times 0.18 mm \times 0.14 μ m with a proprietary stationary phase. An injection at a nominal concentration of 3.2 ng/mL for low-level target pesticides is depicted in Figure 3. This column yielded resolution of all 22 peaks of interest, indication of peak tailing for some species, and significant temperature-dependent baseline drift on the μ ECD. The arrows within the figure point to tailing peaks and highlight baseline drift.

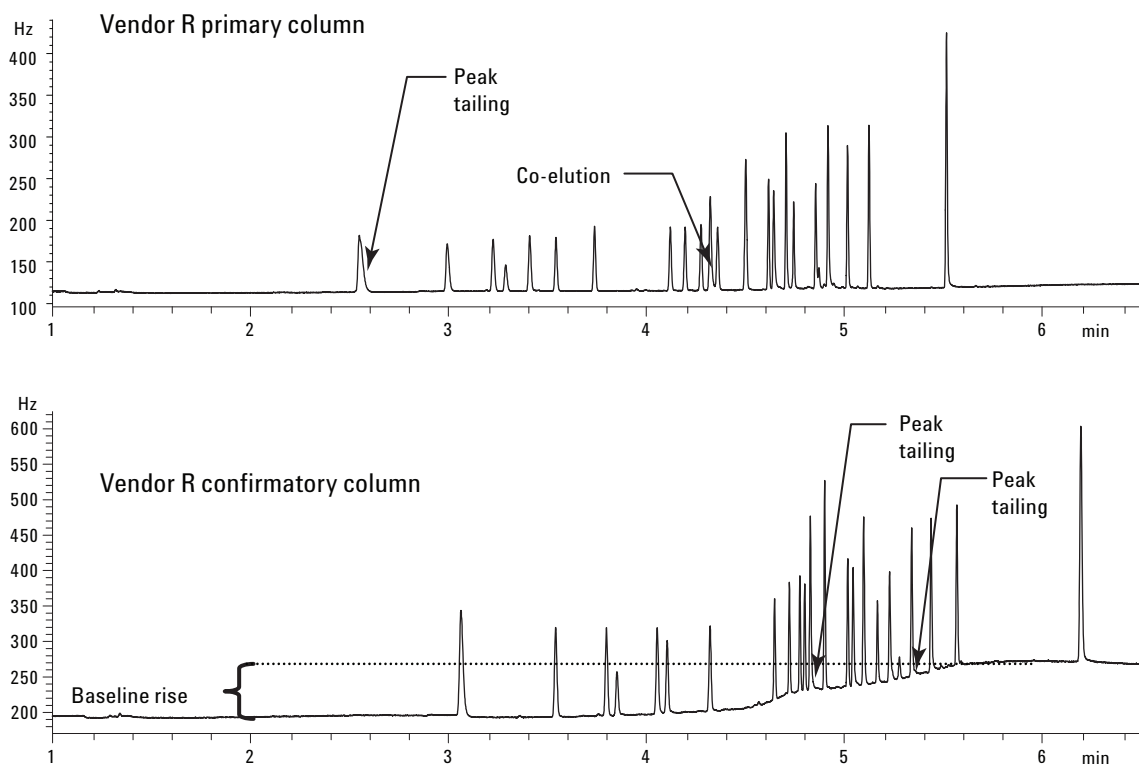


Figure 3. Chromatogram of 0.5- μ L-injection of 3.2 ng/mL low-level target pesticide standard solution injected through a Y-splitter onto Vendor R's primary and confirmatory analysis columns, conditions as in Table 4.

Table 4. Chromatographic Conditions

GC:	Agilent 6890N
Sampler:	Agilent 7683B, 5 μ L syringe (Agilent p/n 5181-1273), 0.5 μ L injection
Carrier:	Hydrogen (flow programmed, 45 cm/s at 120 $^{\circ}$ C, ramped at 99 mL/min to 72 cm/s at 4.4 minutes, purified through a Big Universal Trap (Agilent p/n RMSH-2))
Inlet:	Split/splitless; 220 $^{\circ}$ C pulsed splitless (35 psi for 0.5 min, purge flow 40 mL/min on 1 minute, gas saver 20 mL/min at 3 minutes)
Inlet liner:	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Retention gap:	5 m \times 0.25 mm id deactivated (Agilent p/n 160-2255-5)
Y-splitter:	Quartz deactivated (Agilent p/n 5181-3398)
Columns:	1 20 m \times 0.18 mm \times 0.18 μ m primary analysis column 2 20 m \times 0.18 mm \times 0.14 μ m confirmatory analysis column
Oven:	120 $^{\circ}$ C (0.50 min); 60 $^{\circ}$ C/min to 160 $^{\circ}$ C; 30 $^{\circ}$ C/min to 260 $^{\circ}$ C; 40 $^{\circ}$ C/min to 300 $^{\circ}$ C (2.0 min)
Detection:	μ ECD 320 $^{\circ}$ C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

Conclusions

Agilent's 0.18-mm id primary analysis column is superior to Vendor R's offering. All 22 peaks of interest were resolved on the DB-17ms primary analysis column in less than 6 minutes with sharp, symmetrical peaks and minimal baseline drift. Vendor R's primary analysis column resolved 20 of 22 peaks of interest and displayed evidence of peak tailing for some of the peaks of interest.

Agilent's 0.18-mm id confirmatory analysis column offering is superior to Vendor R's offering. Twenty of 22 peaks of interest were resolved on the DB-XLB, with the other two peaks being almost baseline resolved in less than 6 minutes, with sharp, symmetrical peaks and minimal temperature-dependent baseline drift. Resolution of 20 of 22 peaks is less than ideal but should serve well for peak confirmation. Vendor R's confirmatory column resolved all 22 peaks of interest but showed evidence of peak tailing and an unacceptable level of temperature-dependent baseline drift.

The DB-17ms and the DB-XLB columns used in these experiments gave very low bleed profiles on the μ ECDs. The stable baselines produced by both of these columns can lead to lower detection limits,

simpler integration and more reliable results over time. These columns also have the versatility of use with other analyses beyond CLP pesticides.

Reliable CLP pesticide primary and confirmation analyses are achievable using Agilent J&W high-efficiency GC columns in less than 6 minutes with standard gas chromatographic equipment.

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