Contract Laboratory Program (CLP) Pesticide Analysis with 0.18 mm ID High-Efficiency GC Columns Utilizing Helium Carrier Gas

Application

Environmental

Authors

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Abstract

Contract Laboratory Program (CLP) pesticide analysis is demonstrated on high-efficiency GC columns (20 m × 0.18 mm id × 0.18 µm film thickness) with helium carrier gas. DB-17ms stationary phase is used for primary analysis and DB-XLB stationary phase for confirmation. Primary analysis and confirmation of 22 CLP pesticides in the protocol is achieved in an 11-minute analysis, a 35% reduction in analysis time versus 0.32 mm id columns.

Method translation software is successfully employed to translate an original set of conditions with hydrogen carrier gas to a new set of conditions using helium carrier gas. Elution order and degree of separation are shown to translate precisely from the original method to the new method through use of this software (available for free download) [1].

Introduction

The determination 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, adding costs to both the lab and its client. Here, 0.18 mm id high-efficiency GC columns are demonstrated as a means of enhancing laboratory productivity. These columns are fully compatible with standard gas chromatographs and helium carrier gas operation. The high efficiency these columns offer coupled with their full compatibility with existing GCs provide laboratories with a powerful tool for enhancing sample throughput. When analysis times for 30 m × 0.32 id and 20 m × 0.18 mm id columns were compared, a 17-minute analysis was reduced to only 11 minutes and with improved resolution [2].

Helium carrier gas was selected as a means to show the utility of 0.18 mm id columns in doing CLP pesticide analyses and to demonstrate the full compatibility of these columns with standard gas chromatographs. The operating gas pressures for these 20 m × 0.18 mm id columns range from 33 psi initially to 50 psi at the high point of the temperature program. The gas pressure range used with helium carrier gas with these 0.18 mm id columns was well within the operating range for standard chromatographs.

Experimental

This work was accomplished using an Agilent 6890N GC equipped with dual µECDs and a 7683B autosampler. A single split/splitless injection port was used for sample introduction at the head of a retention gap column connected through a Y-splitter with two analytical columns. Details of the chromatographic conditions are presented in Table 1.

Table 1. Chromatographic Conditions

GC	Agilent 6890N, 240 V Oven
Sampler:	Agilent 7683B, 5 µL syringe (Agilent p/n 5181-1273), 0.5 µL injection
Inlet	Split/splitless; 250 °C pulsed splitless (35 psi for 0.5 min)
Inlet liner	Deactivated single taper direct connect (Agilent p/n 1544-80730)
Carrier	Helium (constant flow, 49.5 cm/sec at 120 °C, purified through big universal trap Agilent p/n RMSH-2)
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 (0.49 min); 85 °C/min to 160 °C; 20 °C/min to 260 °C (0.20 min); 25 °C/min to 285 °C; 40 °C/min to 300 °C (3.5 min)
Detection	µECD 325 °C; nitrogen makeup; constant column + makeup flow 60 (mL/min)

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

Table 2. Flow Path Supplies

		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 loop	430-1020
Tubing cutter	Ceramic wafer column cutter	5181-8836

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 separately into two 100-mL volumetric flasks in 2,2,4 trimethylpentane and then combined in subsequent serial dilutions. Volumetric flasks and pipettes used were all class A. Standard concentration range for low-level target compounds in the protocol was from 1.6 to 40 ng/mL. On-column loading ranged from 0.4 to 10 pgs for low-level target compounds when a 0.5-µL injection over both columns is considered.

Column Installation Tip on 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 loop 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 gave tight, reliable connections that have lasted without any difficulty for more than 2 months (to date) and hundreds of oven temperature program cycles.

Results and Discussion

The starting point for this application was a set of conditions for CLP pesticide analyses using hydrogen carrier gas and 0.18-mm high-efficiency columns developed by Wool and Decker [3]. Using hydrogen carrier and flow programming, they were able to achieve primary separation and confirmation analysis of CLP pesticides in a 7-minute analysis. The chromatographic parameters for the hydrogen carrier separation were input as initial setpoints in method translation software to convert the method to use with helium carrier. Helium carrier was selected for use in laboratories reluctant to work with hydrogen carrier due to site safety policy or individual preference. High-efficiency GC columns give the chromatographer the option to work with either helium or hydrogen carrier gases and still achieve faster analyses.

Wool and Decker [3] indicated in their paper that frequent trimming of the front of the column was necessary for use with heavy matrix samples due primarily to the lower sample capacity of 0.18-mm columns. In this work a 5-m 0.25-mm id retention gap and Y connector were installed ahead of the

analytical columns to help offset the diminished sample capacity relative to wider bore capillary columns. Use of a retention gap will also shield the analytical columns from deleterious matrix affects and extend the useful lives of the columns.

Agilent's method translation software simplifies conversion from established laboratory GC methods to parallel sets of conditions suitable for high-efficiency GC columns. Chromatographic conditions from the original method, along with the new column dimensions, are entered into a menu-driven table within the software. The software then generates a translated method table with all the new chromatographic setpoints for the translated method. The new translated method setpoints produced by the software are often all that is required to successfully translate a method.

Three primary modes of method translation are available in the method translation software: translate only, best efficiency, and fast analysis. The "translate only" mode produces a set of conditions that most closely resembles the original method in terms of relative position on the Van Deemter curve, degree of separation, and elution order. The "best efficiency" mode generates a set of conditions where column efficiency is prioritized. The "fast analysis" mode generates a set of conditions where analysis speed is prioritized. By using the various modes available a translated method specific to a particular application can be developed quickly with a few keystrokes and iterative passes through the software.

The software is very useful in porting methods from the use of one carrier gas to another. Translation from the original method using one carrier to a method using another carrier is accomplished by entering the original method setpoints, the new column dimensions, and the desired carrier. The software then generates the translated method setpoints for the new column and carrier. For additional information on Agilent's method translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.

Flow programming is not addressed in the method translation software, so minor adjustments to flow rate parameters may be required to achieve desired results. When translating flow-programmed methods, initial or intermediate flow rates can be entered into the original method parameters table to visualize the effect on the other parameters' output in the translated method table. The operator can then collect data at several different flow rates and select the best set of conditions for the application.

In this CLP pesticide example, the original method used a hydrogen carrier and flow programming. The initial flow parameters were entered into the method translation software, along with the new column dimensions, specifying helium as the carrier gas. Translate-only mode was selected in the software and produced the translated method setpoints that appear in Figure 1.

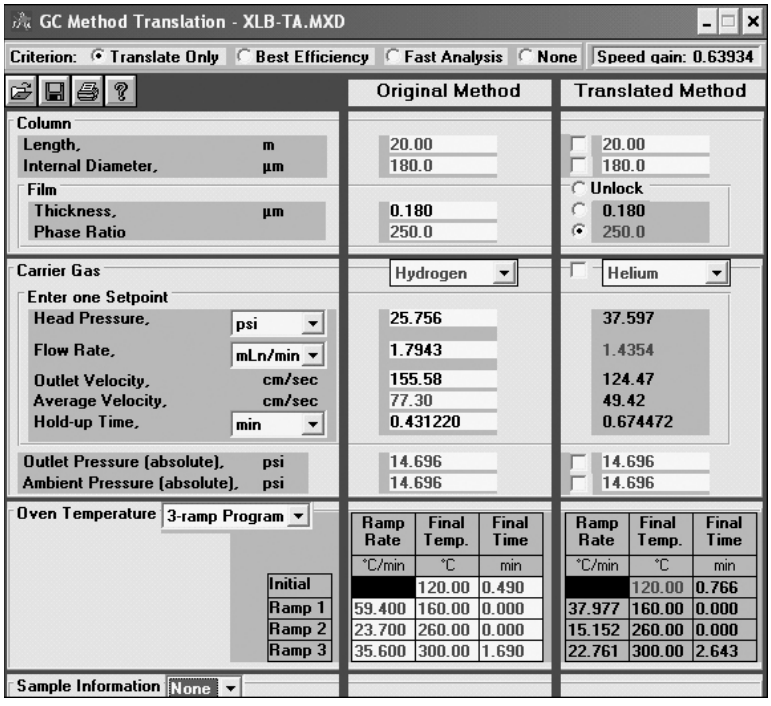

Figure 1. Method translation using translate-only mode.

Figure 2 shows the resulting CLP pesticide separations produced using translate-only mode in the method translation software on the DB-17ms column. Note that all 22 species are baseline resolved on the DB-17ms column where there is a partially separated triplet consisting of gamma chlordane, alpha chlordane, and endosulfan 1 on the DB-XLB column (Figure 3). This partially separated triplet was also observed in the original DB-XLB separation using hydrogen carrier.

Table 3 is a standard compound key for the numbered peaks in the chromatograms. Separation characteristics such as degree of separation and elution order were maintained exactly as they were in the original method using the new translated method with helium carrier. The original method was successfully translated with no additional method development.

Unfortunately, the unresolved triplet on DB-XLB observed in the original method remained unresolved in the translated method. Additional method development attempts focused on resolving the partially separated triplet on the DB-XLB confirmation column and reduction of analysis time. Some success was achieved; however, the trailing two peaks in the triplet remained partially resolved on the DB-XLB confirmation column while analysis time was reduced to 11 minutes. The DB-17ms column resolved all of the species in the protocol throughout these experiments (Figure 4). Triplet resolution on the DB-XLB (Figure 5), though not ideal, is adequate for the purpose of peak confirmation of well-resolved species on the DB-17ms.

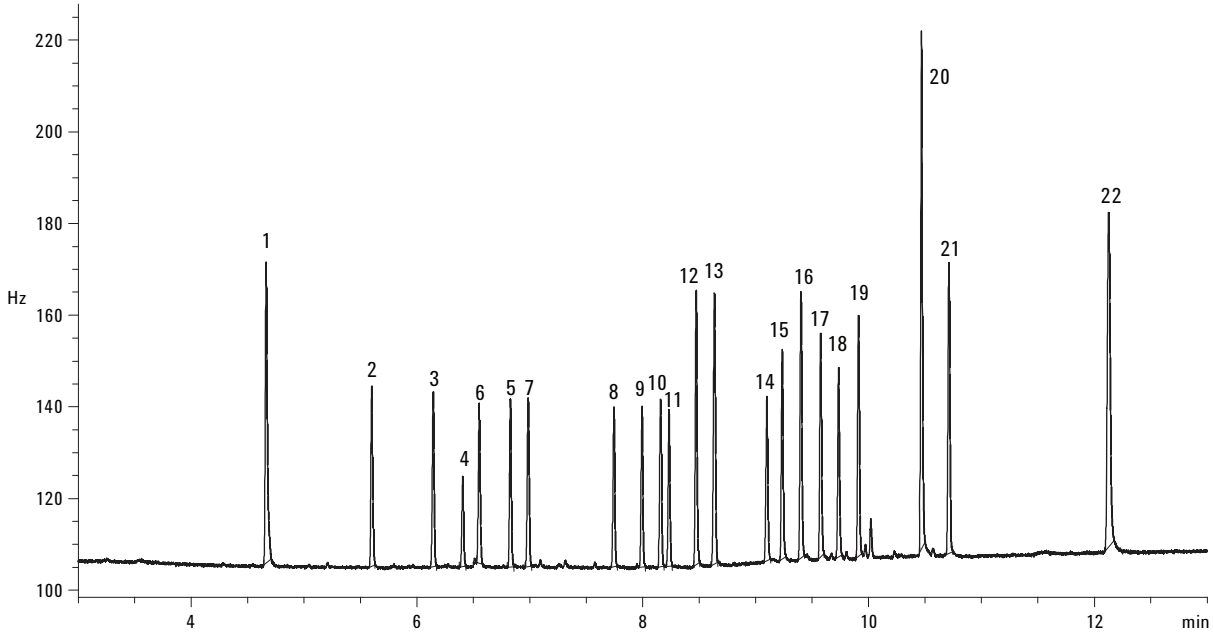

Figure 2. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

Table 3. CLP Standard Compound List Key

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

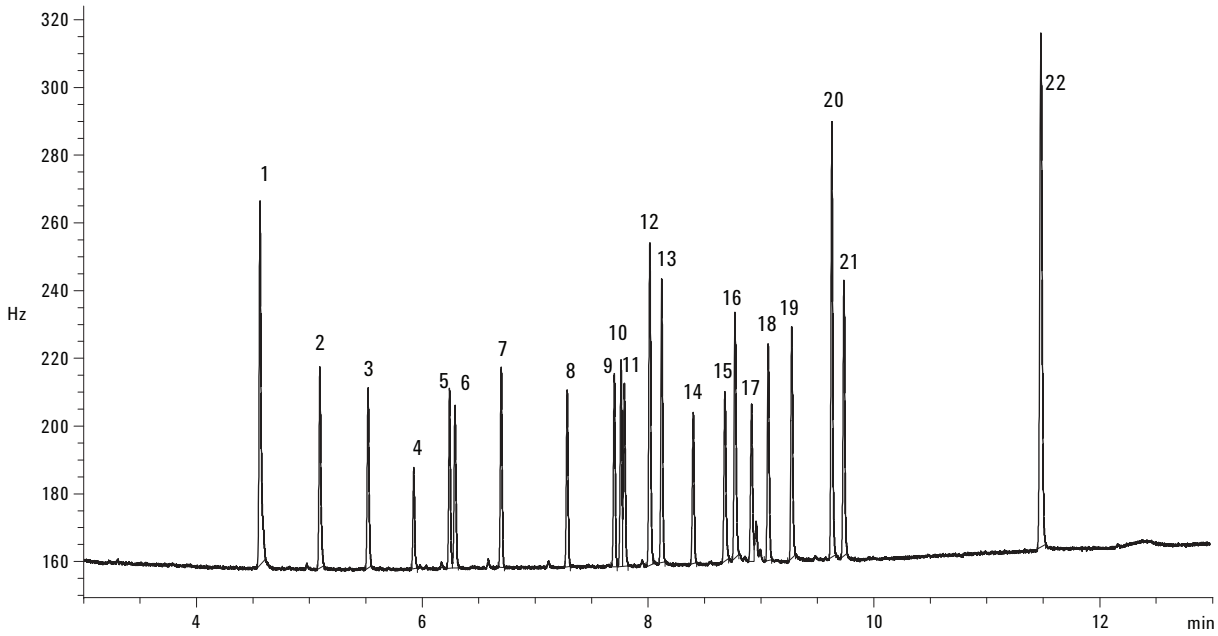

Figure 3. Translate-only separation (conditions as in Figure 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

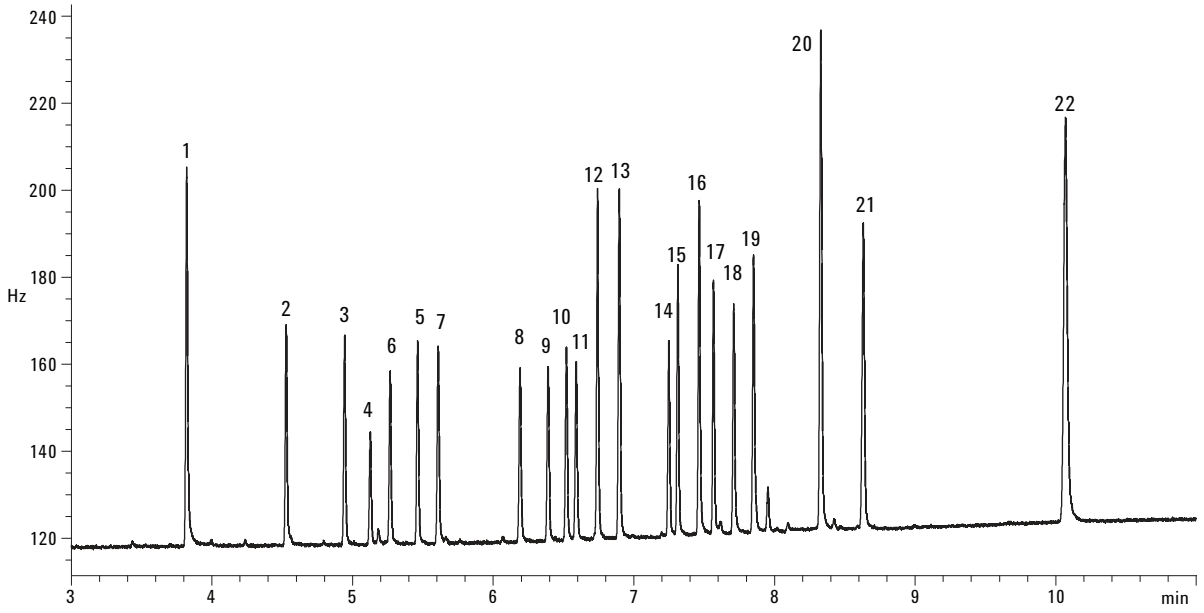

Figure 4. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 0.4 pg/component loading for low-level target compounds.

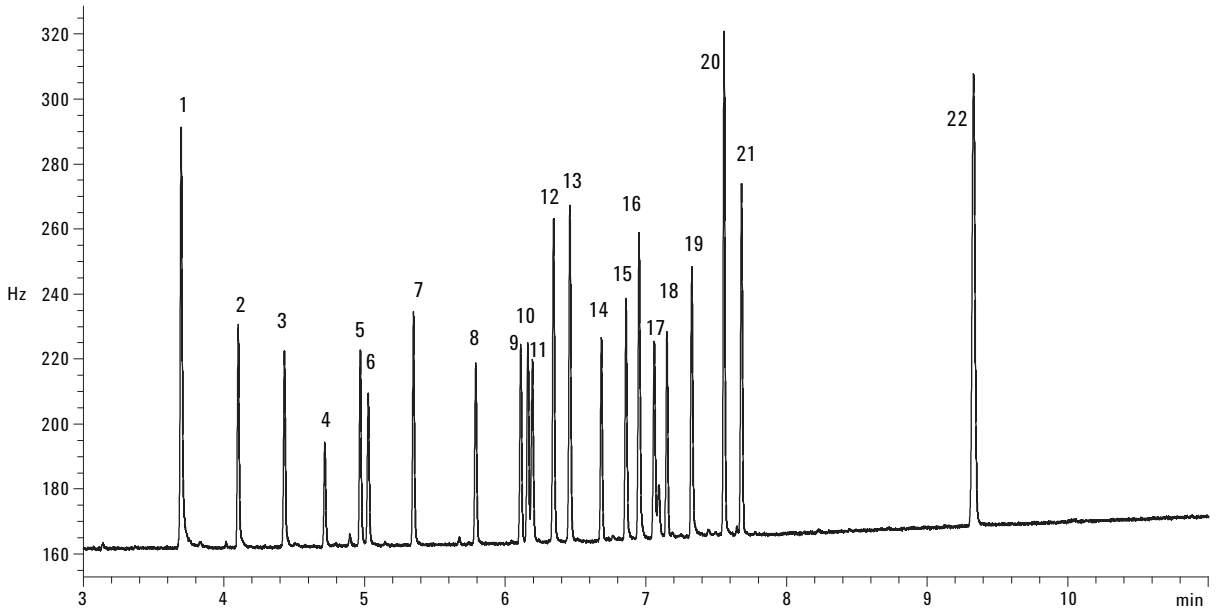

Figure 5. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-XLB (Agilent p/n 121-1222) with a 0.4 pg/component loading for low-level target compounds.

Detector Sensitivity and Linearity

The 0.5-µL injections were split between two columns for an on-column loading of 0.4 pg per component of the low-level target compounds. The data suggest that detection limits of at least an order of magnitude lower are possible. Sensitivity and linearity measurements conducted with these chemical species using µECD detection support this assertion [4]. Analyte concentration range investigated here was from 1.6 – 40 ng/mL. This range meets the 16-fold low- to high-check standard criteria for the protocol and appears to cover only the middle of the dynamic range the detector is capable of fielding. Figure 3 shows the DB-17ms separation where low-level component loading was 0.4 pg. Figure 6 shows the same separation with a 10-pg loading for the same components.

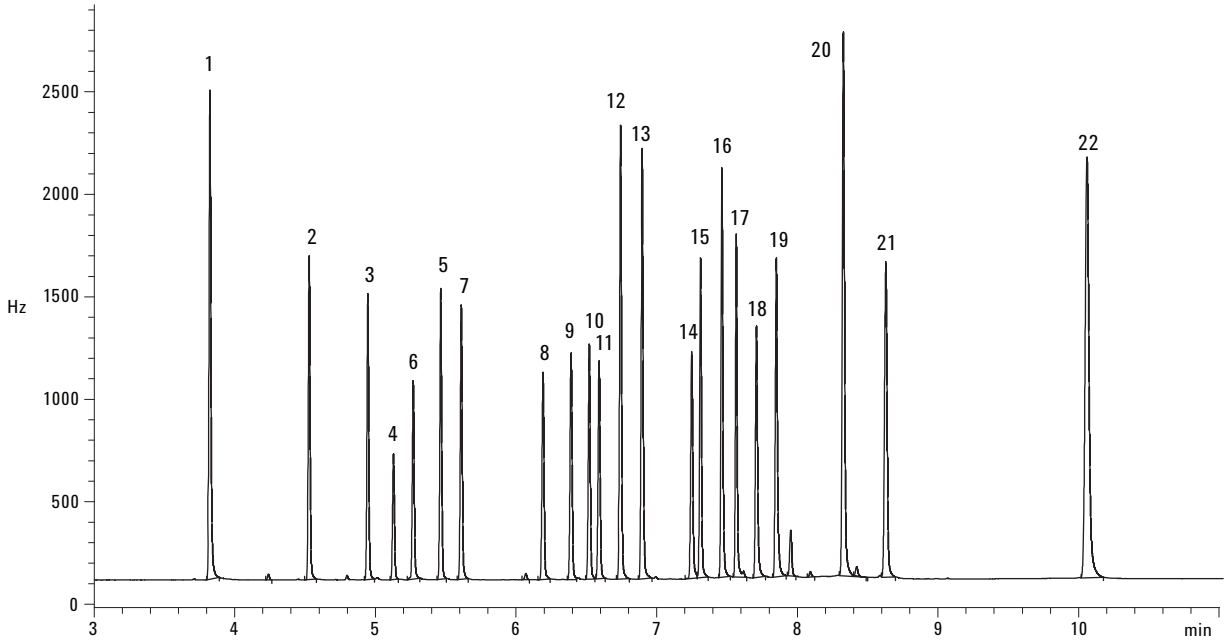

Figure 6. Optimized separation (conditions as in Table 1) on 20 m × 0.18 mm × 0.18 µm DB-17ms (Agilent p/n 121-4722) with a 10-pg/component loading for low-level target compounds.

Conclusions

Complete separation and confirmation of all 22 species in the CLP pesticide protocol were accomplished in an 11-minute analysis with helium carrier gas. These results demonstrate the utility of these 0.18-mm id high-efficiency GC columns for CLP pesticide analysis. Using a 0.5-µL injection of pesticide standard solutions over a concentration range of 1.6 – 40 ng/mL gave excellent results. These results easily meet the 16x high/low dynamic range requirement for the protocol and suggest that expanding the range to both lower and higher concentrations is certainly possible with these 0.18-mm columns.

Full compatibility for use of these columns with standard GC equipment and helium carrier was also established by this successful separation. Operating pressure for use of these columns at the high point of the temperature program (300 °C) was 50 psi, well within the operation pressure range for standard GC equipment.

Method translation software successfully translated the original method using hydrogen carrier to the new method using helium carrier. Separation characteristics from the original method, such as elution order and degree of separation, were matched exactly in the translated method. This exercise served once again to validate the simplicity of method translation using the software. Method development beyond the translated method setpoint only became necessary when improvements to the original separation were attempted.

References

1. To download Agilent Method Translation software, please visit this link: http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm.
2. C. George, “Rapid Analysis of CLP Pesticides Using High-Temperature DB-35ms and DB-XLB Columns,” Application Note 5988-4973EN, December 18, 2001
3. L. Wool and D. Decker, “Practical Fast Gas Chromatography for Contract Laboratory Program Pesticide Analysis,” *Journal of Chromatographic Science,* Vol. 40 September 2002
4. I. L. Chang, M. S. Klee, and J. Murphy, “Validation Analysis of EPA CLP Target Organochlorine Pesticides with the Agilent 6890 Series GC and Micro-ECD,” Application Note 5966-3742E, February 1998

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