The Analysis of Chlorinated Pesticides and PCBs Using the HP-608 Capillary Column

Application Note
228-236

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Abstract

Chlorinated pesticides and PCBs targeted in EPA Methods 608, 8080, 8081, and CLP pesticides for wastewater and solid wastes are analyzed under optimum conditions at a constant flow of 2.4 ml/min. The merits of splitless and on-column injection techniques using the Agilent 5890 Series II GC with electronic pressure control (EPC) are compared.

Key Words: chlorinated pesticides, PCBs, on-column injection, splitless injection, HP-608 capillary column, EPA 608, EPA 8080/8081, CLP pesticides, electronic pressure control.

Introduction

Chlorinated pesticides and PCBs have been banned in the U.S. for several years. However, because of their persistence in the environment, EPA methods 8080/8081 and CLP pesticides target 16 to 20 chlorinated organic pesticides in the evaluation of solid waste. This includes pesticides, their degradation products, technical grades of chlordane, toxaphene, and PCBs in solid waste.[1,2] EPA Method 608 targets similar pesticides in industrial and wastewater discharges.[3] EPA Methods 608 and 8080 prescribe packed-column analysis, whereas Methods 8081 and CLP pesticides prescribe capillary column analysis.

These EPA Methods allow laboratories to substitute columns of their choice provided that performance data such as chromatographic resolution, analyte breakdown, and MDLs (minimum detectable levels) are equal to or better than those provided with the EPA methods.

The HP-608 is a wide bore (530 µm-id) capillary column specially designed for the analysis of organic pesticides. GC/ECD separations of chlorinated pesticides and PCBs were done using the HP-608 column with both on-column and splitless inlet sample introductions. In both cases, the HP-608 provided superior chromatographic resolution, excellent reproducibility, and minimal analyte breakdown for the analysis of pesticides and PCBs.

Experimental

A 30 m x 530 µm x 0.5 µm HP-608 column (part no. 19095S-023) was used under constant carrier gas flow using the 5890 Series II GC with EPC equipped with a split/splitless inlet and a cool on-column inlet. Equipment included the 7673 automatic sampler with tray and the electron capture detector (ECD).

Samples were introduced in both the on-column and splitless modes. The Merlin™ Microseal septum (part no. 5181-8816) was used in the split/splitless inlet to replace the conventional inlet septum. A deactivated tapered glass liner (part no. 5181-3316) was used for all splitless injection runs. GC conditions were controlled using the HP 3365

Table 1. Experimental Conditions

Instrument Requirements	
Gas chromatograph:	Agilent 5890 Series II with EPC
Injection ports:	Split/splitless inlet with temperature and pressure programmable features On-column inlet with temperature and pressure programmable features
Column:	HP-608, 30 m x 530 µm x 0.5 µm (Part number 19095S-023)
Detector:	ECD
Sample introduction:	7673 splitless fast injection On-column injection
Data collection:	3365 ChemStation and HP Vectra 486/133T
Experimental Conditions	
Column:	HP-608, 30 m x 530 µm x 0.5 µm (Part number 19095S-023)
Carrier gas:	He, 20 cm/sec, 2.2 psi at 80°C with EPC under constant flow of 2.4 ml/min
Oven:	First ramp: 80°C (hold 1 min) to 190°C at 30°C/min Second ramp: 190°C to 280°C (hold 1 min) at 6°C/min Third ramp: 280°C to 300°C (hold 2 min) at 20°C/min
Injection:	Splitless: 1 µl, inlet temperature of 250°C On-column: 1 µl oven track for inlet temperature program
Detector:	ECD (330°C), 65 ml/min N_2 makeup, 6 ml/min anode purge
Sample:	Pesticides and PCB standard solutions in isooctane

ChemStation. Data was managed with a HP Vectra PC (486/33T). Instrument parameters and experimental conditions are listed in **Table 1**.

Pesticide solutions containing 16 to 22 components were prepared from the dilution of certified standards (part no. 8500-5873 and 8500-5876, mixes A and B: level 2) with isooctane (pesticide residue grade from Burdick & Jackson). Pesticide standards (part no. 5062-3589), including four vials of 16 EPA-608 pesticides and two vials of two component inlet check solutions (endrin/DDT concentrations are 50 ppb/100 ppb), were used without further dilution. These pesticide compounds are listed in **Table 2**.

Figure 1. Chromatograms of the 16 chlorinated pesticides under optimum GC conditions, 100 pg of each pesticide injected. Peak identification in Table 2.

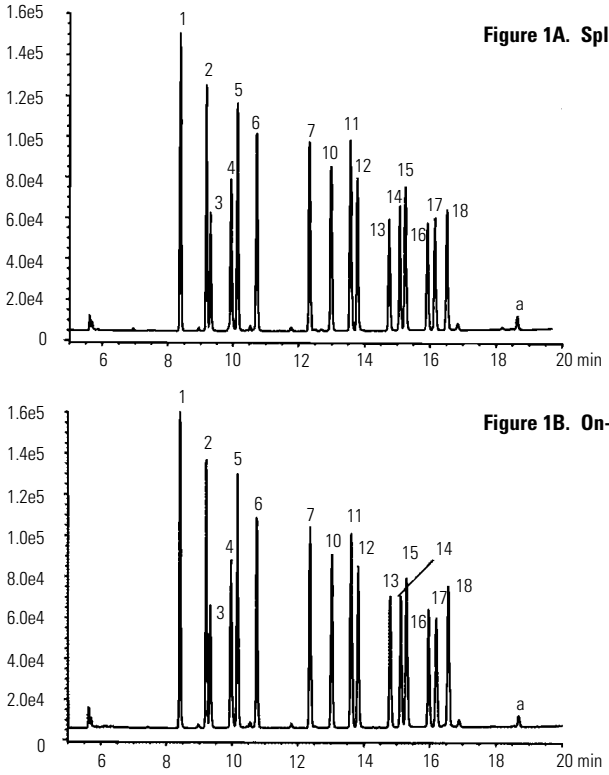


Table 2. Chlorinated Pesticides

Peak No.	EPA-608	Compound Name EPA-8080/8081	EPA-CLP Pesticides
1	alpha-BHC	alpha-BHC	alpha-BHC
2	Lindane	Lindane	Lindane
3	beta-BHC	beta-BHC	beta-BHC
4	Heptachlor	Heptachlor	Heptachlor
5	delta-BHC	delta-BHC	delta-BHC
6	Aldrin	Aldrin	Aldrin
7	Heptachlor epoxide	Heptachlor epoxide	Heptachlor epoxide
8		Chlordane-gamma	Chlordane-gamma
9			Chlordane-alpha
10	Endosulfan I	Endosulfan I	Endosulfan I
11	4,4′-DDE	4,4′-DDE	4,4′-DDE
12	Dieldrin	Dieldrin	Dieldrin
13	Endrin	Endrin	Endrin
14	4,4′-DDD	4,4′-DDD	4,4′-DDD
15	Endosulfan II	Endosulfan II	Endosulfan II
16	4,4′-DDT	4,4′-DDT	4,4′-DDT
17	Endrin aldehyde	Endrin aldehyde	Endrin aldehyde
18	Endosulfan sulfate	Endosulfan sulfate	Endosulfan sulfate
19		Methoxychlor	Methoxychlor
20	a-Degradation product		Endrin ketone
SS1			Tetrachloro-m-xylene
SS2			Decachlorobiphenyl

Results and Discussion

Splitless Analysis

Figure 1A shows the analysis of a standard solution containing the 16 EPA-608 targeted pesticides at a constant column flow of 2.4 ml/minute. One microliter of sample (100 pg of each component) was introduced in splitless mode at 250°C under the conditions[4] listed in **Table 1**. All 16 components were well resolved in sharp symmetric peaks, and the analysis was completed in less than 17 minutes. The 30-m HP-608 (530 µm id) column possesses sufficient efficiency to completely resolve the complex pesticides mix, including chlorinated compounds with similar or isomeric structures. The absence of coeluting peaks on the HP-608 column permitted fast and accurate identification and quantitation.

Low-Temperature On-Column Analysis

Figure 1B shows the same pesticides standard mix using the cool on-column injection technique. On-column injection of 1 µl of sample at 80°C resulted in little sample degradation, minimal byproducts, and good sensitivity (see **Table 3**). Common to both **Figures 1A** and **1B** is the absence of tailing peaks, including the endrin aldehyde peak (peak 17), indicating the HP-608 column surface is very inert.

Reproducibility

Reproducibility for the analysis of chlorinated pesticides using HP-608 columns with the HP GC/ECD system was excellent (see **Table 3**). The RSD (relative standard deviation) in absolute area counts for all 16 EPA targeted pesticides was less than 2% for on-column runs (two sets of six replicate injections). Similarly, the peak area counts reproducibility for all splitless injection runs (three sets of six replicate injections) was in the 1% to 2% RSD range using the same standard sample.

The standard deviation of retention times was within 0.003–0.005 minutes and 0.002 minutes for on-column and splitless runs, respectively. In comparison, the standard deviation of retention times for EPA Method 8081 analysis (**Table 10**, reference 1) using wide-bore capillary columns ranged from 0.007 minutes to 0.013 minutes for the same set of pesticides. This clearly demonstrates that chromatographic reproducibility obtained using the HP-608 capillary column is better than that obtained using the capillary columns stipulated in EPA Method 8081.

Table 3. Reproducibility of Pesticide Analysis

	Retention Times, min			Area Counts		
Pesticides	**Mean**	**Std Dev**	**% RSD**	**Mean**	**Std Dev**	**% RSD**
A. On-column injection (100 pg each component)						
alpha-BHC	8.423	0.004	0.047	431643	7497	1.74
Lindane	9.225	0.004	0.046	393514	6496	1.65
beta-BHC	9.352	0.004	0.046	208287	3428	1.65
Heptachlor	9.984	0.004	0.042	310294	5430	1.75
delta-BHC	10.181	0.005	0.044	390027	7428	1.90
Aldrin	10.760	0.004	0.039	359246	6996	1.95
Heptachlor epoxide	12.385	0.003	0.028	359586	5740	1.60
Endosulfan I	13.036	0.004	0.031	321622	5478	1.70
4,4′-DDE	13.623	0.004	0.026	341930	7070	2.07
Dieldrin	13.838	0.004	0.027	336042	4832	1.44
Endrin	14.814	0.004	0.025	268560	5298	1.97
4,4′-DDD	15.135	0.004	0.024	254389	3017	1.19
Endosulfan II	15.311	0.004	0.025	297580	4326	1.45
4,4′-DDT	15.975	0.003	0.021	259369	3881	1.50
Endrin aldehyde	16.208	0.004	0.022	205588	1876	0.91
Endosulfan sulfate	16.570	0.003	0.021	281397	4143	1.47
a, Degradation product	18.690	0.003	0.017	3416	97	2.83
B. Splitless injection (100 pg each component)						
alpha-BHC	8.351	0.002	0.020	376446	7222	1.92
Lindane	9.146	0.002	0.020	317405	6592	2.08
beta-BHC	9.273	0.002	0.018	165105	3129	1.90
Heptachlor	9.898	0.002	0.018	207924	4637	2.23
delta-BHC	10.097	0.001	0.013	301779	6113	2.03
Aldrin	10.671	0.002	0.015	308689	6422	2.08
Heptachlor epoxide	12.289	0.001	0.011	289985	6216	2.14
Endosulfan I	12.938	0.002	0.014	253489	5496	2.17
4,4′-DDE	13.527	0.001	0.011	313249	6102	1.95
Dieldrin	13.735	0.002	0.014	209054	3925	1.88
Endrin	14.710	0.002	0.013	160235	3104	1.94
4,4′-DDD	15.034	0.002	0.013	168113	3094	1.84
Endosulfan II	15.207	0.002	0.015	228810	4868	2.13
4,4′-DDT	15.874	0.002	0.012	168810	2129	1.26
Endrin aldehyde	16.103	0.002	0.010	148655	3687	2.48
Endosulfan sulfate	16.467	0.002	0.013	190284	3003	1.58
a, Degradation product	18.584	0.002	0.012	21513	1747	8.12

Comparison of Sample Introduction Techniques

For all on-column injection runs, degradation was negligible due to the low initial column temperature (80°C) and the direct introduction of a liquid sample plug into an inert column. As a result, inlet-related sample discrimination, alteration, and degradation were eliminated, while the advantages of solvent focusing and stationary phase focusing were maximized. Routine analysis of the inlet check solution (specified by the EPA methods) showed that the average degradation was less than 3% for endrin and 1% for DDT.

As demonstrated by the clean baseline in **Figure 1A**, little sample degradation occurred at an inlet temperature of 250°C. However, a small endrin ketone peak (RT of 18.69 minutes) appeared on the chromatograms from the GC runs with both on-column and splitless injection shown in **Figures 1A** and **1B**. A closer look (**Table 3**), shows that the area counts for endrin ketone (peak a, a byproduct of endrin degradation) measured 5 times larger in the splitless runs than for the on-column runs (average absolute area counts of 3,400 versus 21,000). The GC runs of the inlet check standard (after 200 repeated splitless injections), showed a 7% endrin degradation and 10% DDT degradation. These values were well below the EPA requirement of 15% degradation for both endrin and DDT.

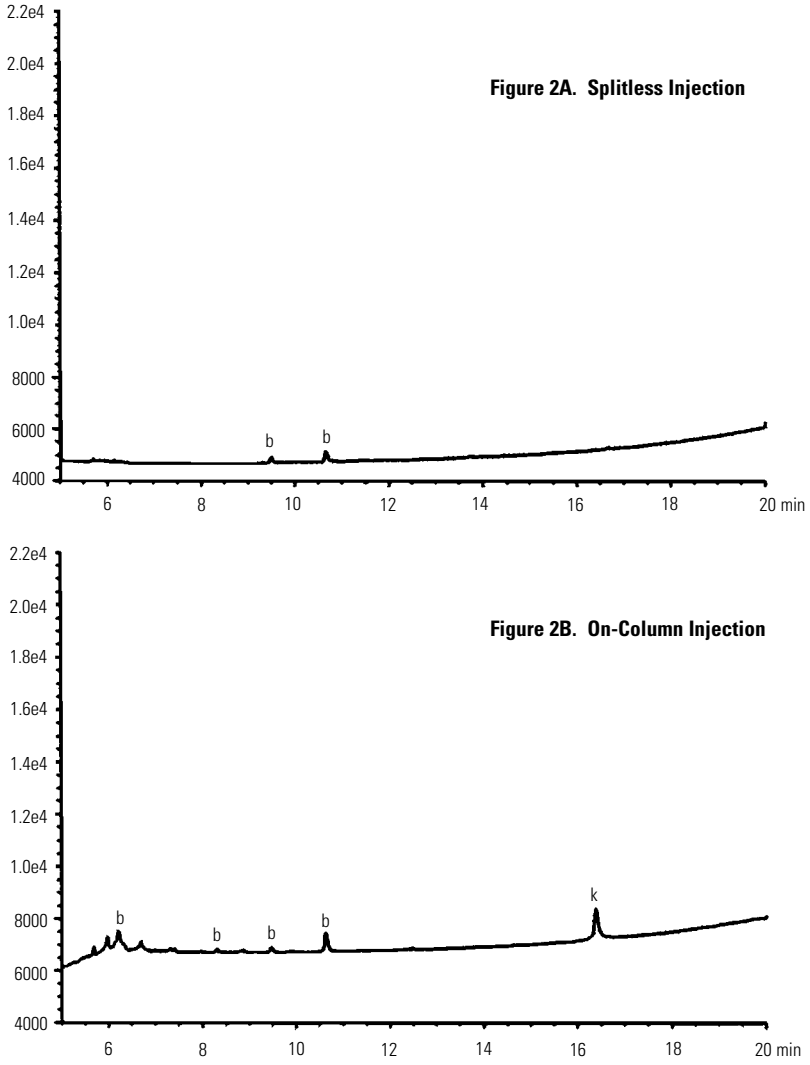
Use of the Merlin™ Microseal[5] and the deactivated glass liner also contributed directly to the low degradation rate in the splitless mode. The Microseal is designed to provide a good inlet seal without using a conventional septum. By eliminating the introduction of particulates into the inlet liner from conventional septum, useful life for the inlet liner is extended, down time (to change a liner and a conventional septum) is reduced, and laboratory throughput is increased.

The use of splitless injection technique may also prevent interference from extraneous and high boiling materials in dirty samples. This is demonstrated in **Figures 2A** and **2B**. **Figure 2** shows the analysis of isooctane solvent (pesticide-residue grade) using both splitless (**Figure 2A**) and on-column injection (**Figure 2B**). The late-eluting peak (peak k) , at 16.69 minutes retention time in the on-column run, does not appear in the chromato-gram of the splitless run (**Figure 2A**).

This peak, possibly a high boiling contaminant in isooctane, appears again in **Figure 3B**. **Figures 3A** and **3B** show analyses of a 10-ppb pesticide standard using splitless injection and on-column injection, respectively. The peak (peak k) eluting just before endosulfan sulfate (peak 18) may cause a higher value for the determination of trace endosulfan sulfate in the sample.

Both area counts and peak heights for the splitless runs were smaller than those for the on-column injection runs (see **Table 3**). For example, the average counts of lindane from the splitless runs were approximately 80% of those from the on-column injections (**Table 3**). Therefore, on-column injection is a good choice for clean samples and trace analyses demanding high sensitivity and low detection limits (large area counts).

Figure 2. Chromatograms of isooctane under optimum GC conditions, 1 µl injected. (b,k=solvent contaminants)



Analysis of PCBs and EPA Methods 8080, 8081, and CLP Pesticides

For wastewater and solid waste samples, the EPA recommends splitless injection for the determination of pesticides and PCBs. Using splitless injection under optimum 5890 Series II GC conditions, all 17 pesticides targeted by EPA Method 8080B are resolved as shown in **Figure 4**.

Among the 20 components targeted by EPA Methods 8081 and CLP pesticides, all but alpha-chlordane and endosulfan I (they are partially separated) are well resolved by the HP-608 column (**Figure 5**). Since the HP-608 column can effectively separate the complex mix of these pesticides, it is a good column choice for the determination of PCBs and multiple-peak response pesticides such as chlordane and toxaphene. **Figure 6** shows a comparison of chromatograms for technical grade chlordane and toxaphene, while **Figure 7** is a comparison of chromatograms for seven PCBs, all analyzed under the same GC conditions using the HP-608 capillary column.

Figure 3. Chromatograms of dilute pesticides mix under optimum GC conditions; 10 pg of each pesticide injected. (Peak ID, see Table 2)

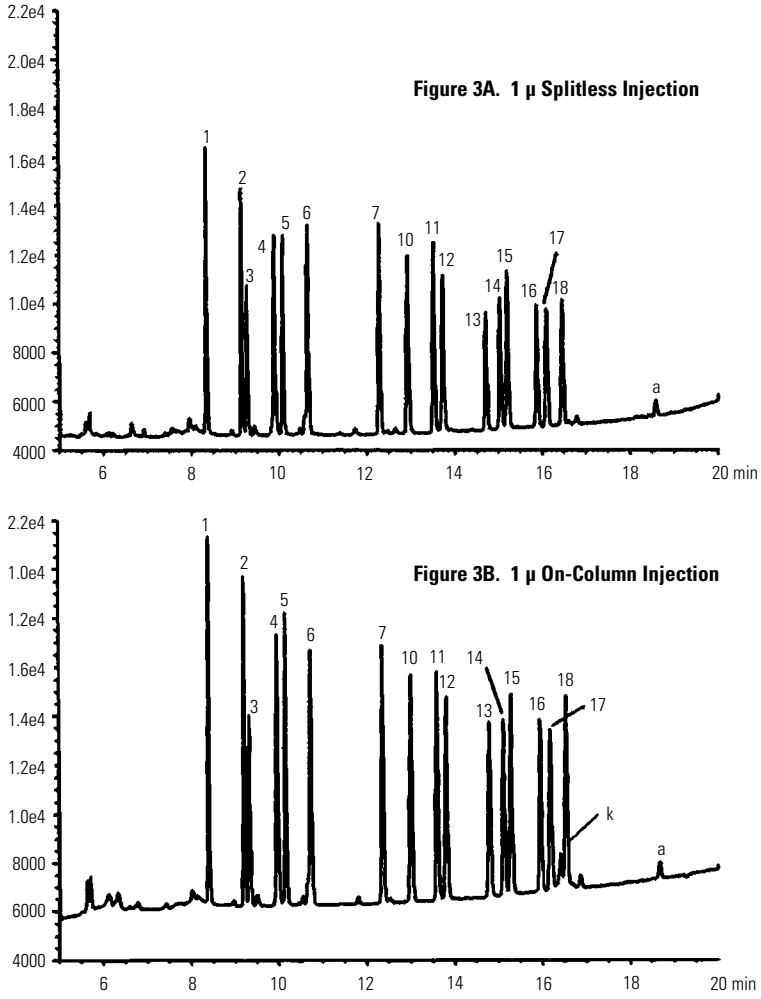
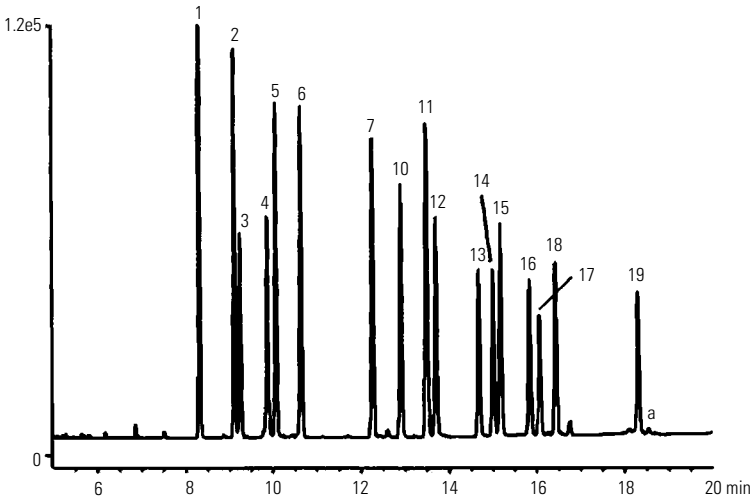


Figure 4. Chromatograms of the EPA-Method 8080 pesticides under optimum GC conditions. Splitless injection of 100–200 pg per component. (Peak ID, see Table 2)



Conclusion

Under optimal conditions, the HP-608 column separates 16 EPA-608 pesticides in 17 minutes and 20 EPA-CLP pesticides (and EPA-8081 pesticides) in 19 minutes (22 minutes including the surrogate, decachlorobiphenyl). Both splitless and on-column injections yield little sample degradation and provide excellent reproducibility of retention times and area responses. On-column injection is more suitable for clean samples and trace analysis, while splitless injection is better used for wastewater and waste samples.

Figure 5. Chromatogram of pesticides targeted in EPA-method 8081 and CLP pesticides under optimum GC conditions. Splitless injection of 50–100 pg per component. (Peak ID, see Table 2)

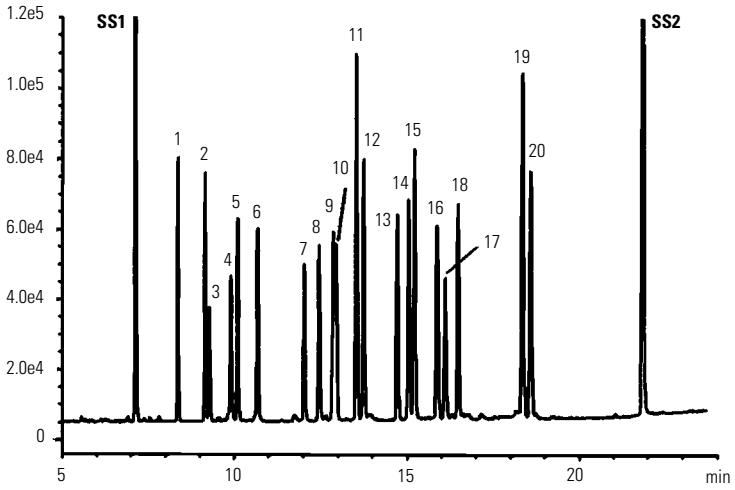


Figure 6. Chromatogram of technical grade toxaphene and chlordane under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm mix

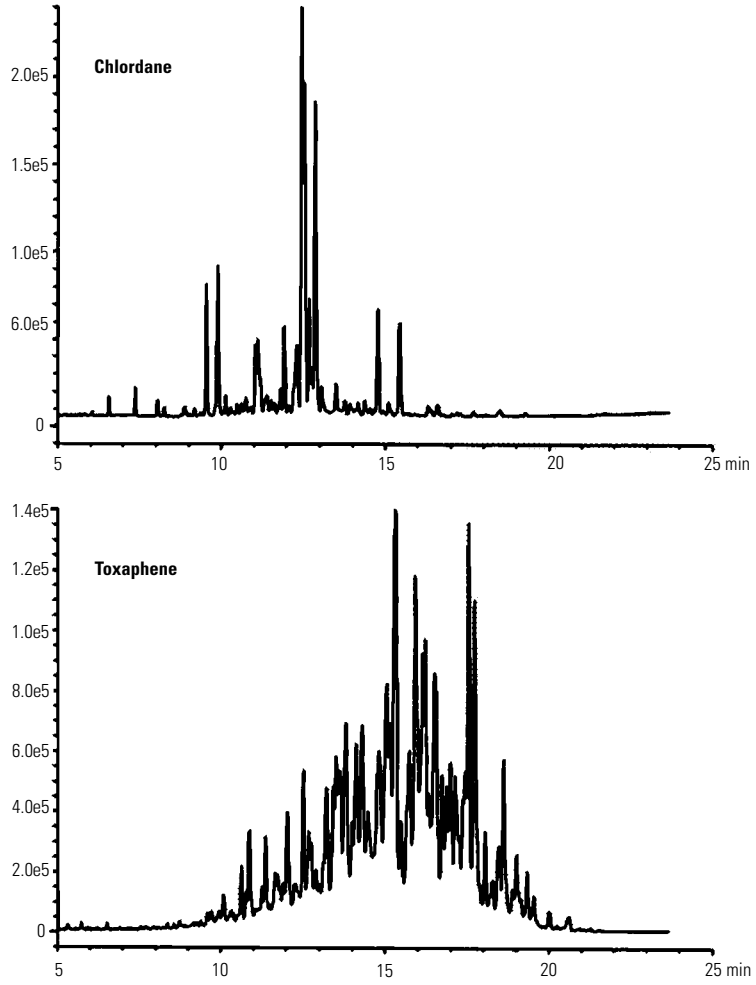
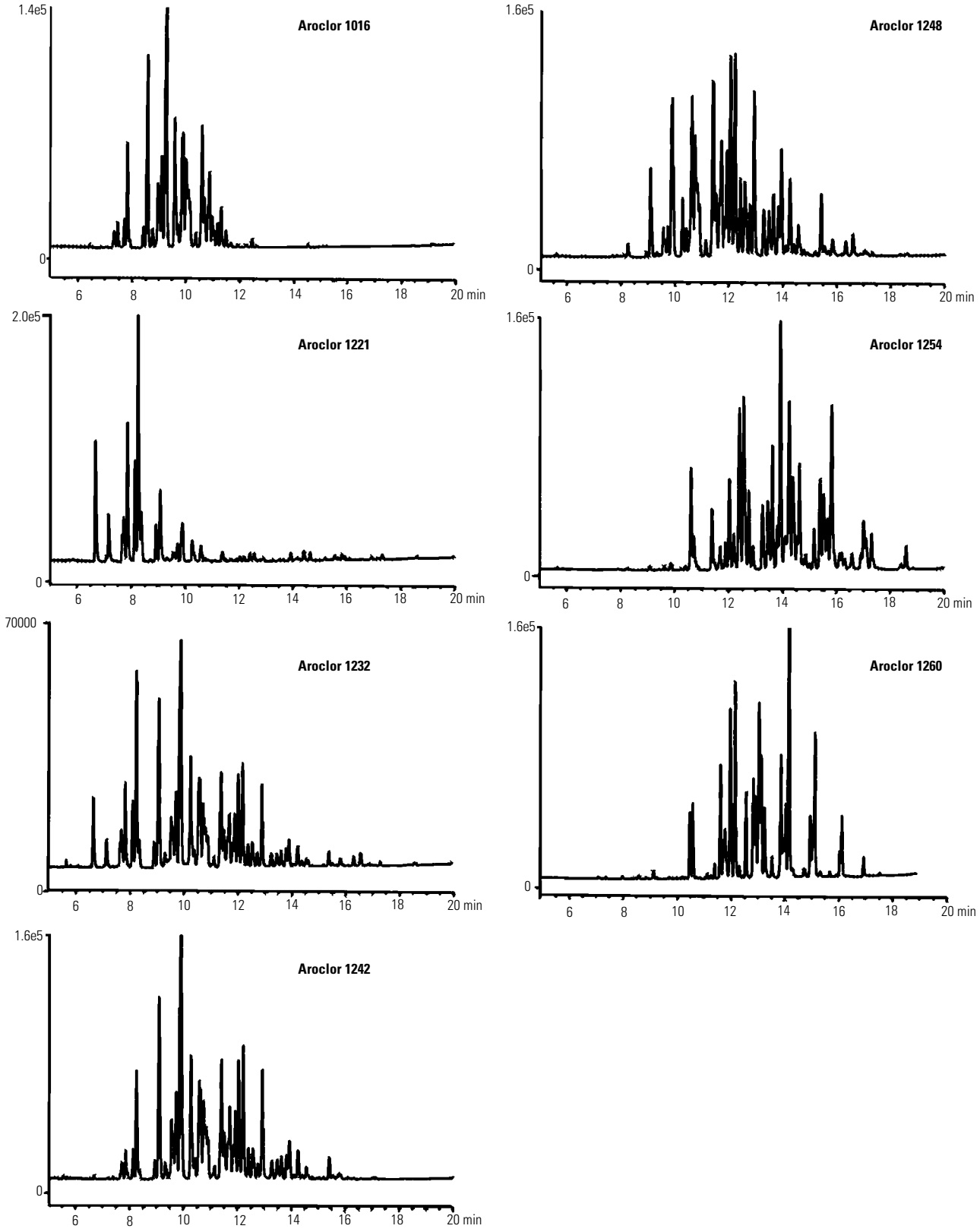


Figure 7. A comparison of seven PCBs under optimum GC conditions. Splitless injection of 1 µl 2.5 ppm each



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