# Identification and Quantitation of PCB Aroclor Mixtures in a Single Run Using the Agilent 7000B Triple Quadrupole GC/MS

Application Note


## Authors

Dale R. Walker and
Fred Feyerherm
Agilent Technologies, Inc.
Santa Clara, CA 95636



## Abstract

A unique method has been developed to enable identification and quantitation of the individual Aroclors present in a mixture in one run, without prior sample cleanup, using MS/MS pattern matching and combined calibration curves. It makes use of guidelines from EPA Methods 8082 and 8270, and the utility of the method was demonstrated using two customer-provided samples of soil and blood.


Agilent Technologies

## Introduction

Traditionally, the analysis for PCB Aroclors, or polychlorinated biphenyls, has been done by gas chromatography/electron capture detector (GC/ECD) methodology. In the past, a single stage gas chromatography/mass spectrometry (GC/MS) system lacked the ability to achieve the detection limits of an ECD due to matrix interferences.

This application note describes a method using the Agilent 7000 Series Triple Quadrupole GC/MS in MS/MS mode to not only achieve, but surpass the selectivity and required detection limits of the traditional GC ECD detector. In addition, the method offers confirmation of each Aroclor in a mixture in a single run, using the individual congener group retention time and MS/MS multiple reaction monitoring (MRM) transition patterns for positive identification of each Aroclor. Using MS/MS enables accurate identification of complex mixtures of Aroclors in difficult matrices by minimizing interferences. The performance of the method was demonstrated using two customer samples: blood and an environmental sample. This GC/MS/MS method thus provides a powerful tool to laboratories that need to perform rapid, accurate, and sensitive (low parts per billion (ppb)) identification and quantitation of mixtures of PCB Aroclors in a range of matrices, in a single run.

## Experimental

### Standards and reagents

| Standard | Source |
|---|---|
| Aroclor 1016, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1221, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1232, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1242, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1248, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1254, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1260, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1262, 1,000 µg/mL in isooctane | Ultra Scientific |
| Aroclor 1268, 1,000 µg/mL in isooctane | Ultra Scientific |
| Acenapthene-$d_{10}$ internal standard | Supelco |

Calibration standards were prepared by serial dilution in isooctane, and adding a consistent amount of the acenapthene-$d_{10}$ internal standard.

### Instruments

This method was developed on the Agilent 7000B Triple Quadrupole GC/MS coupled to the Agilent 7890A Gas Chromatograph using a split/splitless inlet and the Agilent G1544-8070 liner without glass wool. The instrument conditions used are shown in Table 1.

*Table 1. Agilent 7890A Gas Chromatograph and Agilent 7000B Triple Quadrupole Mass Spectrometer Conditions*

| **GC run conditions** | |
|---|---|
| Analytical column | Agilent HP5-MSUI, 30 m × 250 µm, 0.25 µm (p/n G3900-63001) |
| Injection volume | 1 µL |
| Inlet | Agilent MMI, liner without glass wool (p/n G1544-8070) |
| Injection mode | Splitless |
| Inlet temperature | 250 °C |
| Average velocity | 23.498 cm/s |
| Oven temperature | 70 °C for 1.5 minutes,<br>16 °C/min to 200 °C,<br>hold for 1.5 minutes |
| Carrier gas | Helium in constant flow mode at 1 mL/min |
| Transfer line temperature | 280 °C |
| Run time | 10.125 minutes |
| **MS conditions** | |
| Acquisition mode | Electron ionization (EI),<br>multiple reaction monitoring (MRM) |
| Collision gas | Constant flow, 2.5 mL/min |
| Quench gas | Constant flow, 1.5 mL/min |
| MS temperatures | Source: 280 °C<br>Quadrupole: 300 °C |

## Analysis parameters

The transitions used to detect and quantitate the PCB Aroclors are given in Table 2.

*Table 2. MRM Transitions Used to Identify and Quantitate Aroclors*

| Aroclor | Quantifier transition (*m/z*) | Qualifier transitions (*m/z*) | Dwell (msec) | Collision energy (V) |
|---|---|---|---|---|
| 1,016 | 258 → 186 | 222 → 152 | 20 | 25 |
| | | 291.9 → 222 | 20 | 25 |
| 1,221 | 188 → 152 | 222 → 152 | 20 | 25 |
| | | 258 → 186 | 20 | 25 |
| 1,232 | 222 → 152 | 188 → 152 | 20 | 25 |
| | | 258 → 186 | 20 | 25 |
| | | 291.9 → 222 | 20 | 25 |
| 1,242 | 258 → 186 | 222 → 152 | 20 | 25 |
| | | 291.9 → 222 | 20 | 25 |
| 1,248 | 291.9 → 222 | 258 → 186 | 20 | 25 |
| | | 325.9 → 255.9 | 20 | 25 |
| 1,254 | 325.9 → 255.9 | 291.9 → 222 | 20 | 25 |
| | | 359.8 → 289.9 | 20 | 25 |
| 1,260 | 359.8 → 289.9 | 325.9 → 255.9 | 20 | 25 |
| | | 393.8 → 323.9 | 20 | 25 |
| 1,262 | 359.8 → 289.9 | 393.8 → 323.9 | 20 | 25 |
| | | 325.9 → 255.9 | 20 | 25 |
| 1,268 | 427.8 → 357.8 | 461.7 → 391.8 | 20 | 25 |
| | | 393.8 → 323.9 | 20 | 25 |
| | | 497.7 → 427.7 | 20 | 25 |

# Results and Discussion

## Aroclor composition

Polychlorinated biphenyls were synthesized primarily for use as coolants and insulating fluids in electrical equipment. Aroclors are mixtures of biphenyls called congeners that are chlorinated to different extents (for example, mono-, di-, or trichlorinated). The toxicity of PCBs varies considerably among congeners. There are many types of Aroclors, and each has a distinguishing suffix number that indicates the degree of chlorination. The first two digits generally refer to the number of carbon atoms in the phenyl rings (for PCBs this is 12). The second two numbers indicate the percentage of chlorine by mass in the mixture. For example, the name Aroclor 1254 means that the mixture contains approximately 54% chlorine by weight.

## Detection and quantitation of the aroclors

The congener compositions of the PCB Aroclors are complex, and vary significantly [1]. These differences can be exploited to definitively identify a given Aroclor (Table 3). It is important to note that the most abundant homologue groups of congeners are the di- and tri-chlorinated bipehenyls for the Aroclors with low percentages of chlorination (1016 and 1242). In contrast, penta-chlorinated PCBs are more abundant in the Aroclors with the highest chlorination percentages (1248, 1254, and 1260), and tetra-chlorinated PCBs are abundant in Aroclors with both low and high degrees of chlorination.

*Table 3. Typical PCB Aroclor Composition by Congener Class*

| Congener homologue class (level of chlorination) | Aroclor | | | | |
|---|---|---|---|---|---|
| | 1016 | 1242 | 1248 | 1254 | 1260 |
| Mono | 0.7%* | 0.8% | 0% | 0% | 0% |
| Di | 17.5 | 15.0 | 0.4 | 0.2 | 0.1 |
| Tri | 54.7 | 44.9 | 22.0 | 1.3 | 0.2 |
| Tetra | 26.6 | 32.6 | 56.6 | 16.4 | 0.5 |
| Penta | 0.5 | 6.4 | 18.6 | 53 | 8.6 |
| Hexa | 0 | 0.3 | 2.0 | 26.8 | 43.4 |
| Hepta | 0 | 0 | 0.6 | 2.7 | 38.5 |
| Octa | 0 | 0 | 0 | 0 | 8.3 |
| Nona | 0 | 0 | 0 | 0 | 0.7 |
| Deca | 0 | 0 | 0 | 0 | 0 |

*Percentage of the total chlorine in the Aroclor contained in this congener

These differences in composition of the Aroclors by homologue group form the basis for distinguishing between Aroclors, as each homologue group has an MS/MS transition that can be used to help distinguish it from the others (Table 4). Therefore, each Aroclor can have a unique set of transitions that can help distinguish it from the other Aroclors.

*Table 4. Transitions to Distinguish the PCB Homologue Groups*

| Homologue group | Transition (*m/z*) |
|---|---|
| Deca | 497.7 → 427.7 |
| Nona | 461.7 → 391.7 |
| Octa | 427.8 → 357.8 |
| Hepta | 393.8 → 323.9 |
| Hexa | 359.8 → 289.9 |
| Penta | 325.9 → 255.9 |
| Tetra | 291.9 → 222 |
| Tri | 258 → 186 |
| Di | 222 → 152 |
| Mono | 188 → 152 |

A single time segment containing multiple transitions was used to monitor the transitions of the mono- through nona-chlorinated congener homologues. Agilent MassHunter Workstation software was then used to construct a multipoint calibration curve for each Aroclor standard, using concentrations of 25, 50, 100, 200, and 400 ng/mL. The Relative Response Factor (RRF) method was used to determine the amount of each Aroclor present in the sample, using an internal standard. Mass quantitation was based on the sum of all relevant transitions for the particular Aroclor (Table 2). The Aroclors often had coeluting peaks. In these instances, a subset of the total peak area of each Aroclor that is specific to that Aroclor was used for identification and quantitation.

## Unique multiple calibration curve table

The results from the individual calibration curves were then merged into a single calibration curve table using MassHunter. This table was used in a unique way to identify and quantitate each Aroclor in a mixture of Aroclors. Each Aroclor standard has a specific combination of quantifier ions and qualifier ions, and the quantifier to qualifier ion peak area ratios are also specific. These ratios are stored in the calibration curve table with an acceptable range. If the ratio falls within that range it is shown in light blue. If the ratio is too high, it is shown in red, and if it is too low, it is shown in dark blue (Figure 1). Due to interferences, these ratios may fall outside the limits set in the method.

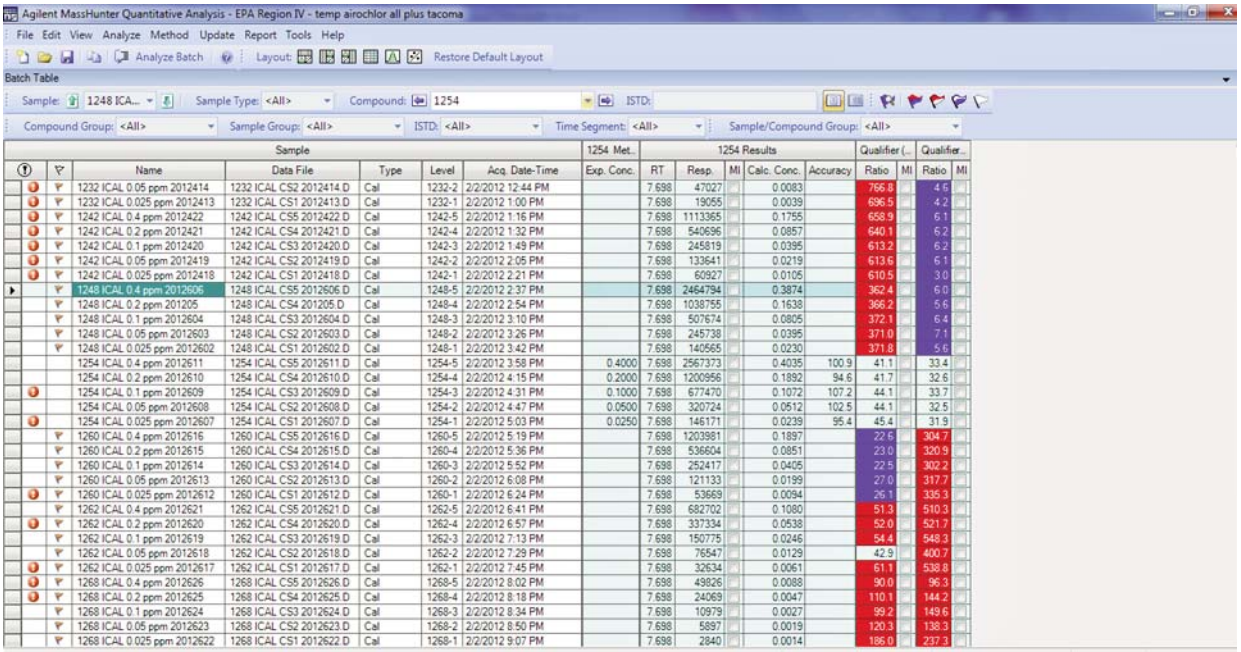



*Figure 1. Combined calibration curve used to identify and quantitate Aroclors.*

If the Aroclor is present, it will meet the criteria set by the curve table, and the qualifier ratios will fall within the parameters set for that Aroclor. For example, Figure 1 illustrates the results from a run of the Aroclor 1254 standard. All of the ratios fall within the allowable range for Aroclor 1254. However, since no other Aroclors are present, all of the other ion ratios show as being out of range, either in dark blue or red. In this case, it is clear that only Aroclor 1254 is present. Interpretation becomes more difficult with Aroclor mixtures, as seen in the examples to follow. The ion ratio limits are not designed to exclude a homologue class, but rather are designed to prompt further investigation by the analyst.

## Environmental sample

An unknown sample received from an environmental site was analyzed to compare the results to those obtained using GC/ECD. The ECD showed such heavy interferences that a positive identification was not possible (Figure 2). In spite of these interferences, a positive identification was made for Aroclor 1248 using the GC/MS/MS method. The total ion chromatogram (TIC) is shown for the unknown in Figure 3.

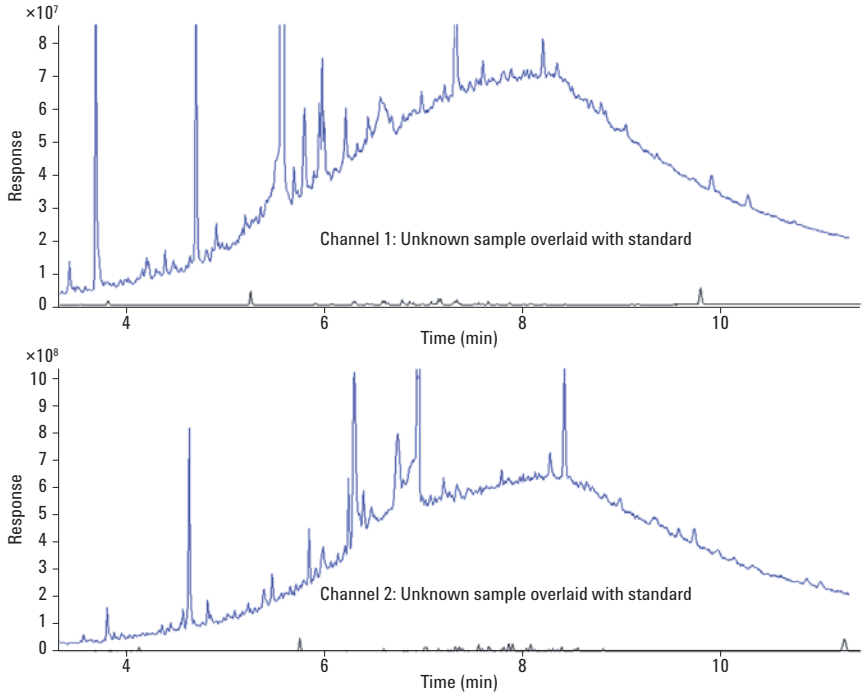



*Figure 2. GC/ECD trace of an Aroclor sample spiked with a standard. The heavy interferences make identification of the Aroclors in the sample impossible, using either channel.*

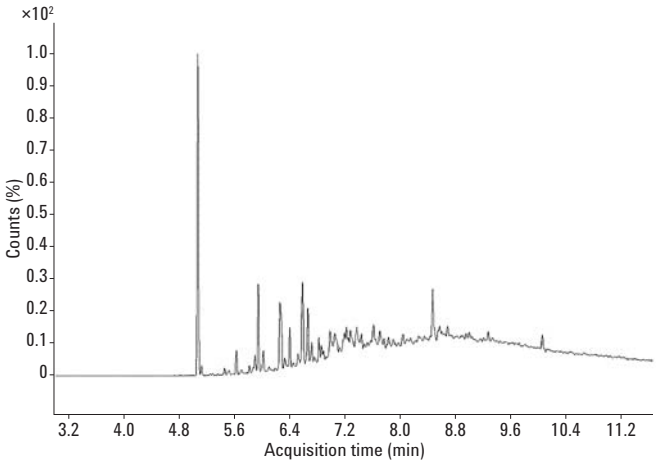



*Figure 3. Total ion chromatogram (TIC) for the environmental sample.*

It is quite complex, and it is not immediately obvious which Aroclor(s) might be present. Due to the amount of significant matrix interference, neither the primary nor secondary qualifier ion fell within the acceptable limits for any Aroclor. However, looking at the four individual transitions that are used to identify Aroclor 1248, it is quite clear that the patterns for those transitions in the standard (Figure 4, E–H), match with patterns seen for the same transitions in the unknown sample (Figure 4, A–D). Thus, the MS/MS method was able to isolate the Aroclor and positively identify it as Aroclor 1248. Using the combined calibration curve (Figure 5), the concentration reported for Aroclor 1248 was 1.407 ppb in the sample, even though the qualifier ratios were out of range, due to interference. Figure 5 also shows the segment of the TIC for the sample that again demonstrates the presence of Aroclor 1248 in the sample. This is an ideal example of positive identification of a compound using multiple transitions and pattern recognition, based on the analyst's experience and expertise.

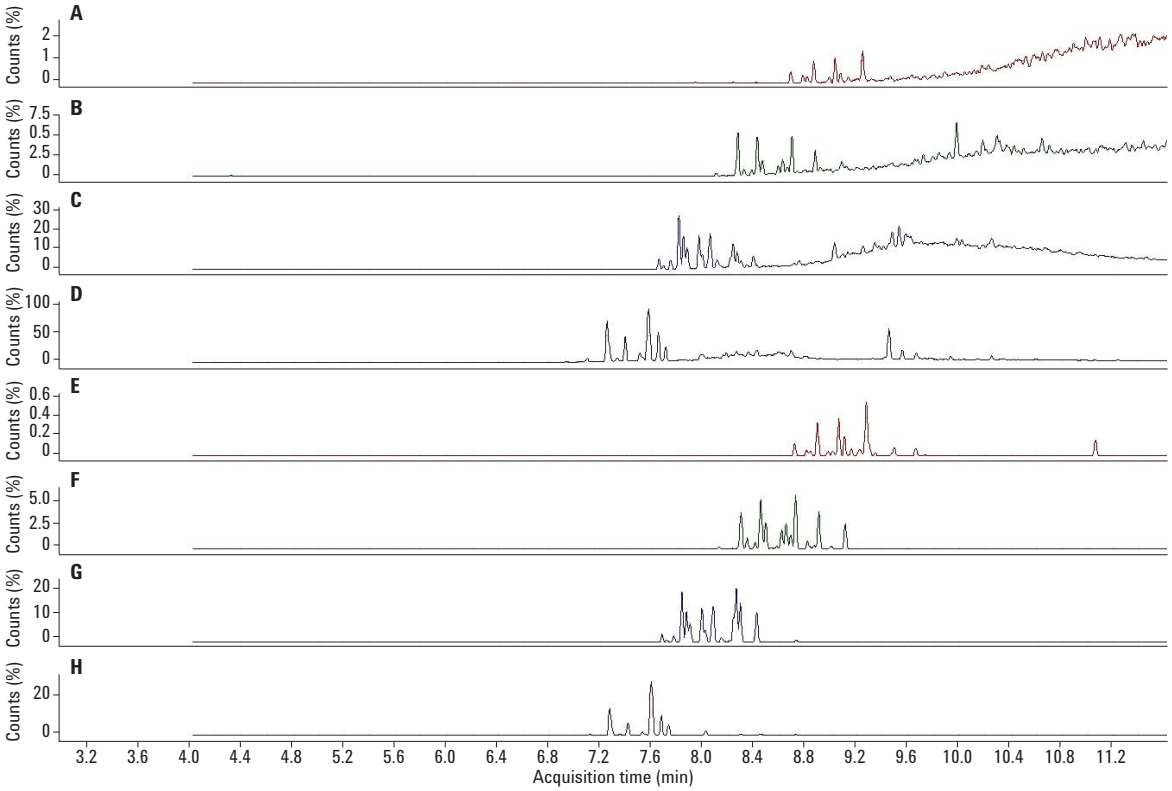



*Figure 4. MRM traces for the four transitions used to identify Aroclor 1248. A–D are the environmental sample, and E–H are the Aroclor 1248 standard. The patterns displayed in the standard are clearly visible in the sample, confirming the presence of Aroclor 1248.*

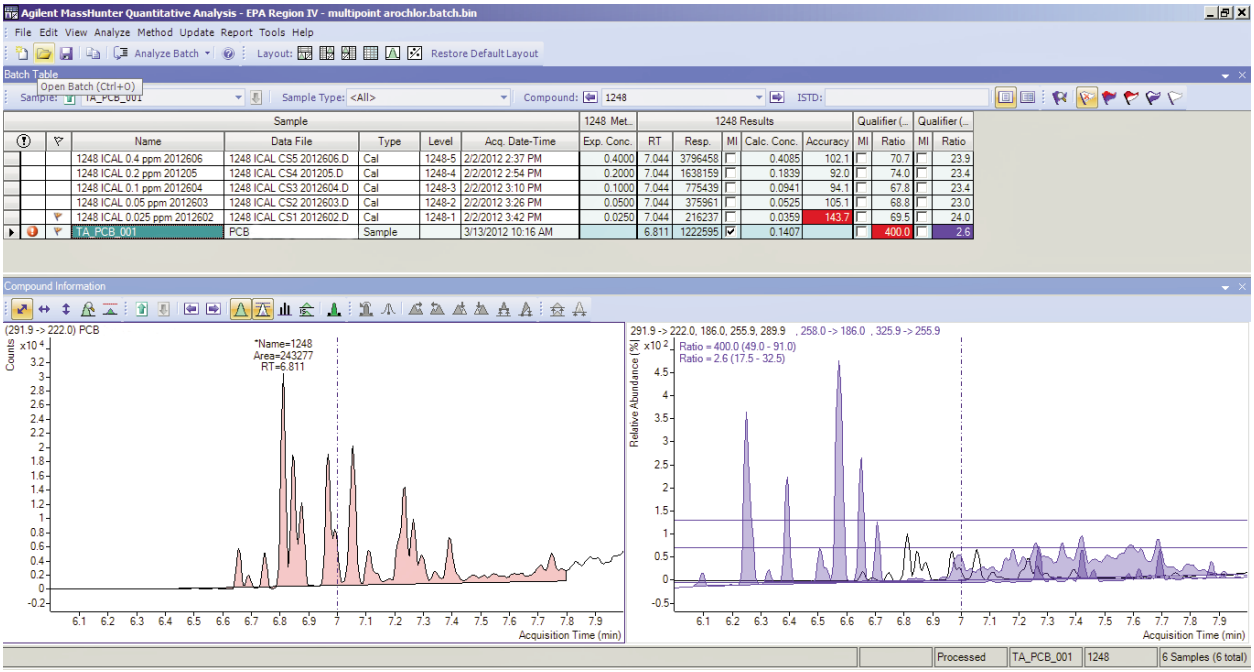



*Figure 5. Combined curve values for the Aroclor 1248 standard and the environmental sample, giving a concentration in the sample of 1.407 ppb. That segment of the TIC indicating the presence of Aroclor 1248 in the sample is also shown.*

## Extracted blood samples

A customer provided two blood extract samples that contained Aroclors of unknown identity spiked at two different concentrations. The samples were analyzed using the method as previously outlined, and the combined curve table was used to interpret the results.

Using the Aroclor 1254 transitions, the sample (bottom two rows) gave a primary qualifier ratio that was too high, but a secondary qualifier ratio that was within range (Figure 6). This pattern was also seen with the Aroclor 1260 standard, using the Aroclor 1254 transitions, suggesting that the sample could contain both Aroclor 1254 and Aroclor 1260.

Agilent MassHunter Quantitative Analysis - Quest Arochlors - quest arochlors.batch.bin

File Edit View Analyze Method Update Report Tools Help

Batch Table

Sample: 05022012-06 Sample Type: <All> Compound: 1254

| | | Data File | Type | Level | Acq. Date-Time | 1254 Met... Exp. Conc. | 1254 Results RT | Resp. | Calc. Conc. | Accuracy | Qua... Ratio | Qua... Ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | arochlor 1254-1.D | Cal | 1254-1 | 5/2/2012 11:11 AM | 5.0000 | 8.048 | 10168 | 8.1266 | 162.5 | 24.5 | 30.8 |
| | | arochlor 1254-2.D | Cal | 1254-2 | 5/2/2012 11:26 AM | 10.0000 | 8.048 | 14319 | 11.4437 | 114.4 | 27.5 | 45.7 |
| | | arochlor 1254-3.D | Cal | 1254-3 | 5/2/2012 11:40 AM | 25.0000 | 8.048 | 37529 | 29.9936 | 120.0 | 28.6 | 43.5 |
| | | arochlor 1254-4.D | Cal | 1254-4 | 5/2/2012 11:55 AM | 50.0000 | 8.048 | 58467 | 46.7277 | 93.5 | 28.7 | 45.0 |
| | | arochlor 1254-5.D | Cal | 1254-5 | 5/2/2012 12:10 PM | 100.0000 | 8.048 | 117251 | 93.7084 | 93.7 | 28.2 | 44.3 |
| | | arochlor 1260-1.D | Cal | 1260-1 | 5/2/2012 12:25 PM | | 8.048 | 5235 | 4.1839 | | 192... | 19.9 |
| | | arochlor 1260-3.D | Cal | 1260-3 | 5/2/2012 12:55 PM | | 8.048 | 25406 | 20.3047 | | 201... | 24.1 |
| | | arochlor 1260-4.D | Cal | 1260-4 | 5/2/2012 1:10 PM | | 8.048 | 43591 | 34.8385 | | 165... | 25.3 |
| | | arochlor 1260-5.D | Cal | 1260-5 | 5/2/2012 1:25 PM | | 8.048 | 82763 | 66.1449 | | 170... | 24.4 |
| | | arochlor1260-02a.D | Cal | 1260-2 | 5/2/2012 1:40 PM | | 8.048 | 8726 | 6.9736 | | 188... | 21.0 |
| | | PCB Data 1.D | Samp... | | 5/14/2012 5:00 PM | | 8.048 | 232 | 0.1858 | | 333... | 30.9 |
| | | pcb data 2.D | Samp... | | 5/14/2012 5:15 PM | | 8.048 | 5310 | 4.2436 | | 114... | 24.2 |
| | | pcb data 3.D | Samp... | | 5/14/2012 5:30 PM | | 8.048 | 53976 | 43.1385 | | 109... | 24.0 |

*Figure 6. Combined calibration curve table showing the results for the analysis of Aroclor 1254 in the blood sample (bottom two rows). The calculated concentrations of the 5 and 50 ppb spiked samples were 4.24 and 43.14 ppb, respectively.*

To confirm the presence of these two Aroclors, the TIC for the sample was examined. Comparing the sample TIC (Figure 7A), with the individual MRM traces for the four transitions characteristic of the Aroclor 1254 standard (Figure 7B), it is quite clear that many of the peaks in the sample are derived from Aroclor 1254. Comparing the sample TIC with the MRM traces for Aroclor 1260 indicated its presence as well (Figure 8)

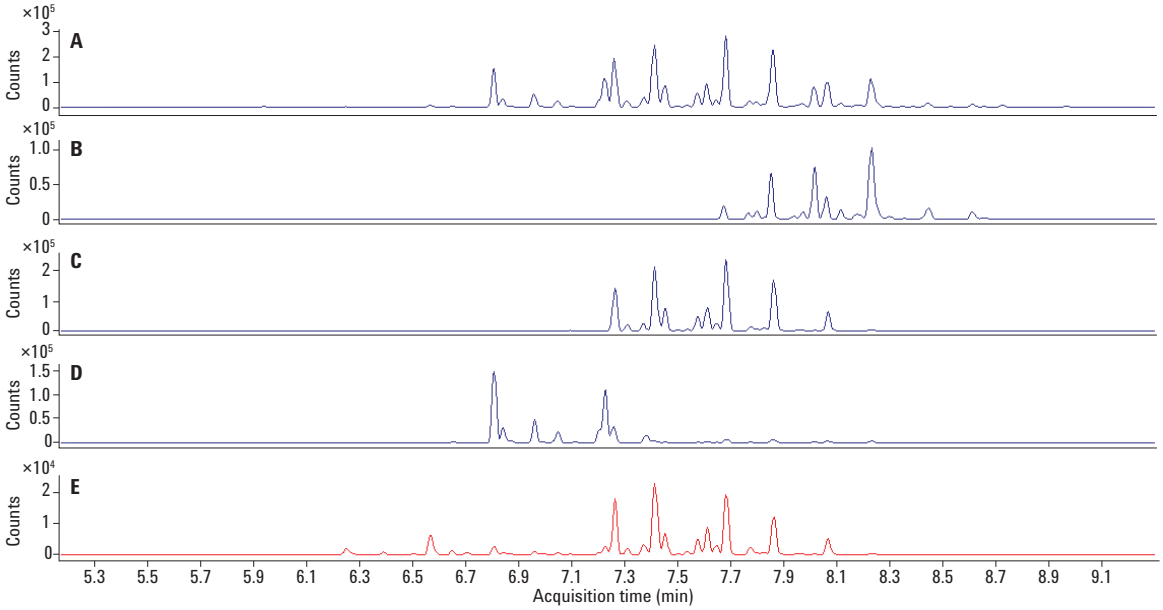



*Figure 7. Comparison of the TIC for the unknown blood sample with four transitions characteristic of Aroclor 1254 confirms the presence of Aroclor 1254 in the sample.*

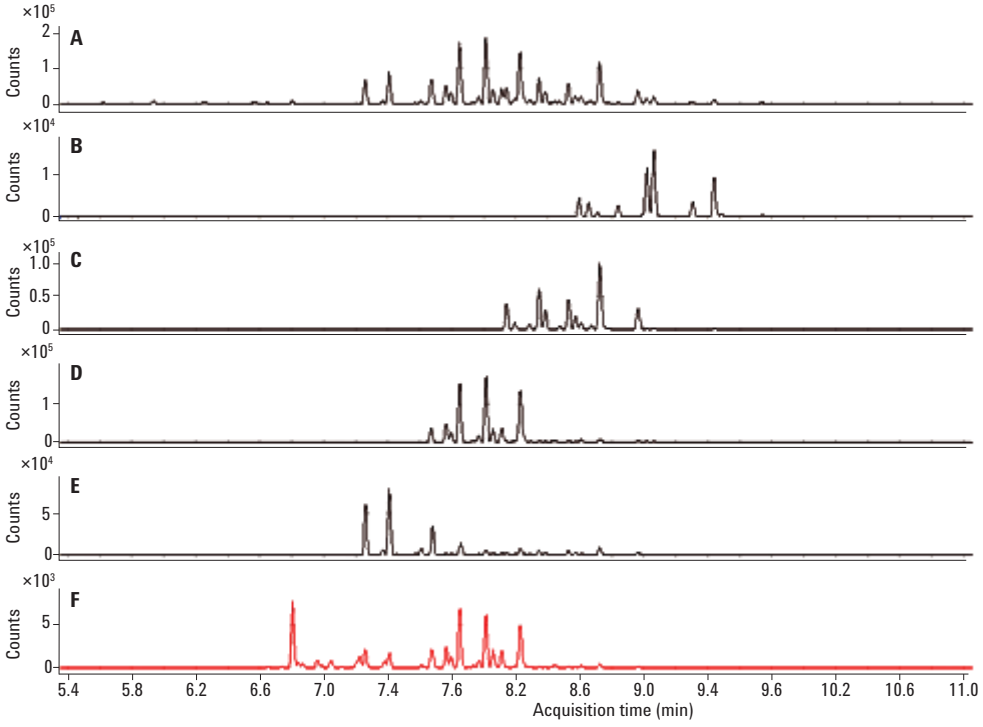



*Figure 8. Comparison of the TIC for the unknown blood sample (A) with four transitions characteristic of Aroclor 1260 (B–F) confirms the presence of Aroclor 1260 in the sample.*

Looking at a subset of three of the transitions used in Figures 8 and 9, and comparing both the Araclor 1254 standard and the Araclor 1260 standard to the sample, it is clear that Araclor 1260 is a component of the sample, as all the peaks in the Araclor 1260 standard are represented in the sample (Figure 9, B and D). However, many of the Araclor 1260 peaks overlap the Araclor 1254 peaks, making it difficult to distinguish the two. The arrows in Figure 9 A indicate some peaks that distinguish Araclor 1254 from Araclor 1260. These peaks are also present in the sample, confirming the presence of Aroclor 1254.

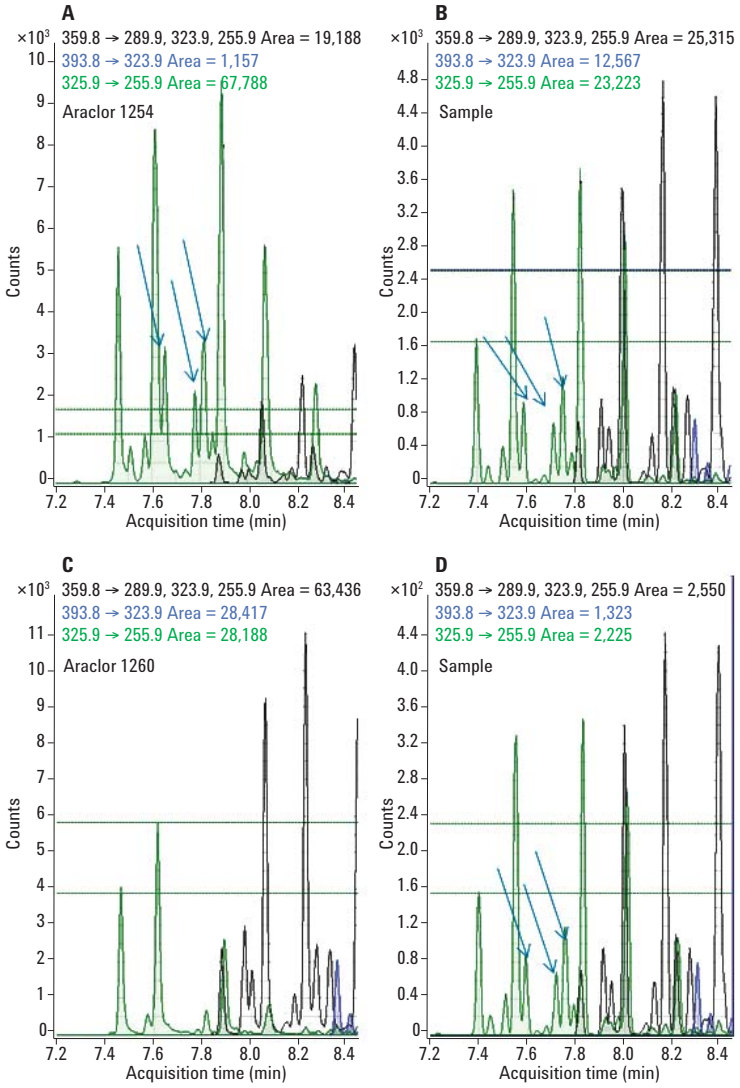
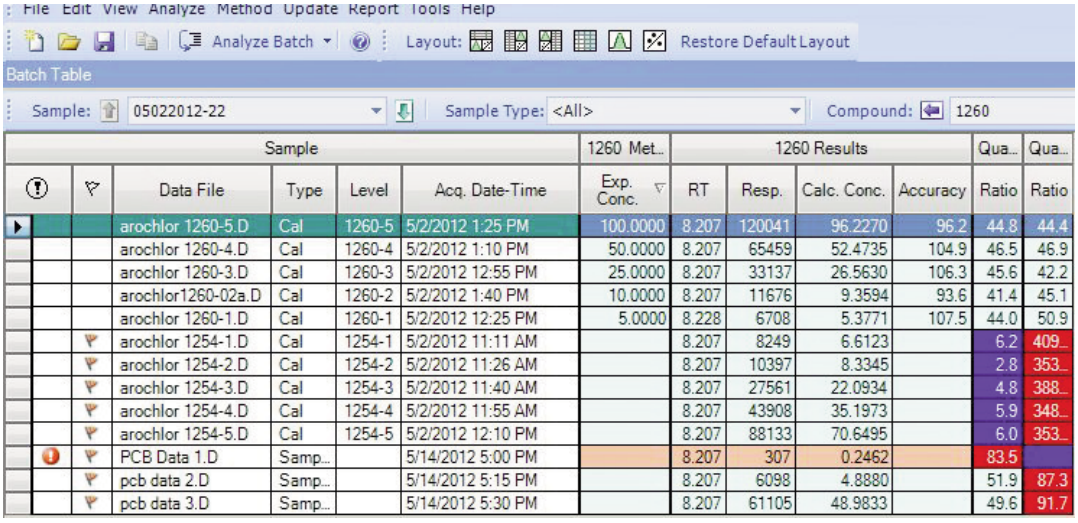



*Figure 9. MRM traces for three transitions used to distinguish Aroclor 1254 from 1260, from analyses of the Aroclor 1254 and 1260 standards, as well as the blood sample. The sample clearly contains both Aroclors, as shown by the unique Aroclor 1254 peaks in the sample (arrows), as well as the overlap of the Aroclor 1260 trace with the sample.*

Once the presence of the two Aroclors was confirmed, the curve table was used to determine the concentration of each Aroclor in each sample. The calculated concentrations were 4.24 ppb and 43.14 ppb for Aroclor 1254 (Figure 6), and 4.89 and 48.98 ppb for Aroclor 1260 (Figure 10). The spiked concentrations were 5 and 50 ppb for each Aroclor.

It is important to note that the presence of Aroclor 1260 was confirmed, and its calculated concentrations were accurate, in spite of the fact that the second qualifier ion ratios were too high (Figure 9). This ratio was too high because of contributions of the same qualifier ion from Aroclor 1254. The successful use of this method requires both the combined calibration table and pattern matching, as well as the expertise and the training of the analyst to recognize when to use qualifier ion ratio data that appears to be out of range.




| Sample | | | | | | 1260 Met... | 1260 Results | | | | Qua... | Qua... |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Data File | Type | Level | Acq. Date-Time | Exp. Conc. | RT | Resp. | Calc. Conc. | Accuracy | Ratio | Ratio |
| | | arochlor 1260-5.D | Cal | 1260-5 | 5/2/2012 1:25 PM | 100.0000 | 8.207 | 120041 | 96.2270 | 96.2 | 44.8 | 44.4 |
| | | arochlor 1260-4.D | Cal | 1260-4 | 5/2/2012 1:10 PM | 50.0000 | 8.207 | 65459 | 52.4735 | 104.9 | 46.5 | 46.9 |
| | | arochlor 1260-3.D | Cal | 1260-3 | 5/2/2012 12:55 PM | 25.0000 | 8.207 | 33137 | 26.5630 | 106.3 | 45.6 | 42.2 |
| | | arochlor1260-02a.D | Cal | 1260-2 | 5/2/2012 1:40 PM | 10.0000 | 8.207 | 11676 | 9.3594 | 93.6 | 41.4 | 45.1 |
| | | arochlor 1260-1.D | Cal | 1260-1 | 5/2/2012 12:25 PM | 5.0000 | 8.228 | 6708 | 5.3771 | 107.5 | 44.0 | 50.9 |
| | | arochlor 1254-1.D | Cal | 1254-1 | 5/2/2012 11:11 AM | | 8.207 | 8249 | 6.6123 | | 6.2 | 409... |
| | | arochlor 1254-2.D | Cal | 1254-2 | 5/2/2012 11:26 AM | | 8.207 | 10397 | 8.3345 | | 2.8 | 353... |
| | | arochlor 1254-3.D | Cal | 1254-3 | 5/2/2012 11:40 AM | | 8.207 | 27561 | 22.0934 | | 4.8 | 388... |
| | | arochlor 1254-4.D | Cal | 1254-4 | 5/2/2012 11:55 AM | | 8.207 | 43908 | 35.1973 | | 5.9 | 348... |
| | | arochlor 1254-5.D | Cal | 1254-5 | 5/2/2012 12:10 PM | | 8.207 | 88133 | 70.6495 | | 6.0 | 353... |
| | | PCB Data 1.D | Samp... | | 5/14/2012 5:00 PM | | 8.207 | 307 | 0.2462 | | 83.5 | |
| | | pcb data 2.D | Samp... | | 5/14/2012 5:15 PM | | 8.207 | 6098 | 4.8880 | | 51.9 | 87.3 |
| | | pcb data 3.D | Samp... | | 5/14/2012 5:30 PM | | 8.207 | 61105 | 48.9833 | | 49.6 | 91.7 |

*Figure 10. Combined calibration curve showing the analysis of Aroclor 1260 in the blood samples (bottom two rows). The calculated concentrations of the 5 and 50 ppb spiked samples were 4.89 and 48.98 ppb, respectively.*

## Conclusions

The Agilent 7000B Triple Quadrupole GC/MS provides a powerful platform for overcoming the limitations of analysis of mixtures of PCB Aroclors using GC/ECD. Using MS/MS and MRM eliminates the interferences that make GC/ECD ineffective. The method developed in this study enables the sensitive identification and quantitation of the components of Aroclor mixtures in a single GC/MS/MS run, using a unique, combined calibration curve table, the MRM patterns for each Aroclor, and the experience and expertise of the analyst to make positive identification, saving time and reducing costs.

## Reference

1. M. Frame, J. W. Cochran, S.S. Bøwadt. "Complete PCB congener distributions for 17 aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis." *J. High Res. Chromatog.* **19**, 657–668 (1996).

## For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem


Printed in the USA
December 5, 2013
5991-3537EN

Agilent Technologies