GCxGC-TOFMS and GCxGC-ECD OF PBDEs

Jack Cochran[1], Frank L. Dorman[2], Eric Reiner[3], Terry Kolic[3], and Karen MacPherson[3]

1 LECO Corporation, 815 Pilot Road, Suite C, Las Vegas, Nevada, 89119
2 Restek Corporation, 110 Benner Circle, Bellefonte, Pennsylvania, 16823-8812
3 Ministry of the Environment, 125 Resources Road, Toronto, Ontario M9P 3V6

Introduction

The polybrominated diphenyl ethers (PBDEs) used as flame-retardants in a wide variety of household and industrial products are by now well-known environmental contaminants[1]. PBDEs are produced commercially as mixtures containing relatively few major congeners and these are the ones often monitored in analytical schemes. Decabromodiphenyl ether (BDE 209) is the most widely used PBDE flame-retardant in the world[2] and it is this congener that complicates the gas chromatography-mass spectrometry (GC-MS) analysis of PBDEs, as it is has a high molecular weight, is nonvolatile, and can thermally degrade. In some labs, the analysis of BDE 209 is accomplished separately (using a shorter GC column) from the other commonly analyzed tri- to heptabromo- BDEs[3,4].

The group of Björklund is one of the most active in documenting solutions to PBDE GC analysis problems, including investigation of injection systems[5] and columns[6] to maximize response for BDE 209 and permit its simultaneous GC analysis with the rest of the PBDE congeners. On-column injection showed the highest yield for PBDEs, but they recommended temperature programmed, pulsed splitless injection for dirty samples. Their column study suggested short, inert, thin film columns should be used for the higher molecular weight PBDEs, but the low capacity of these columns could be problematic for dirtier samples.

For detection of PBDEs, the electron capture detector (ECD), ion trap and quadrupole MS (low resolution), and high resolution (HR) MS with electron ionization and electron capture negative ionization have been employed[5-10]. For quadrupoles and HRMS, selected ion recording is often used to increase sensitivity and selectivity. MS/MS experiments can accomplish this for the ion trap. Recently, we applied vacuum-outlet GC with a 0.53mm column and TOFMS to the analysis of seventeen PBDEs in biosolids, including BDE 209, although the limit of detection for 209 was relatively high[11].

A relatively new way to solve separation problems is to use comprehensive two-dimensional GC (GCxGC). GCxGC is a way to increase peak capacity by applying two independent separations to a sample in one analysis. Typically, GCxGC involves a serial column configuration (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually

(and quickly) focused and "injected" onto the second column. By keeping the second column short, a series of high-speed chromatograms are generated, and the first column separation can be maintained. Separation results can be plotted as a retention plane (column 1 time x column 2 time), also known as a contour plot.

Due to modulation, most GCxGC peaks are on the order of 100 to 500 ms wide, requiring a fast detector. When MS is used, only time-of-flight (TOF) has the necessary acquisition rates (hundreds of spectra/sec). The ability of the thermal modulator to narrow peaks (thereby increasing their height) prior to their detection also affords the ability to increase TOFMS sensitivity for all PBDEs, but especially the difficult 209 congener, without using a secondary column. In this case, only the first dimension column and the TOFMS provide selectivity from sample matrix components.

A study was conducted to evaluate GCxGC-TOFMS for the analysis of PBDEs. Also investigated was the effect of thermal focusing on and reinjection to an uncoated, deactivated transfer line to the TOFMS to improve sensitivity for BDE 209. Finally, a new GCxGC-ECD system was tested for PBDE analysis.

Materials and Methods

PBDE standard solutions were obtained either from Wellington Laboratories (Guelph, Ontario, Canada) or Cambridge Isotope Laboratories (Andover, Massachusetts, USA). Separations were carried out using a LECO Pegasus 4D GCxGC-TOFMS and a LECO GCxGC-ECD system, both with quad jet, dual stage modulators (St. Joseph, Michigan, USA).

For the experiment to increase TOFMS sensitivity for decabromodiphenyl ether by thermal focus/reinject, the GC column configuration was a 10m x 0.18mm x 0.18µm DB-5 from J&W Scientific (Folsom, California, USA) installed in the split/splitless injector of an Agilent 6890 GC (Wilmington, Delaware, USA), connected by a press-fit to a 1m x 0.25mm piece of IP-Deact (Restek Corporation, Bellefonte, Pennsylvania, USA). The uncoated, but deactivated 0.25mm tubing passed through the thermal modulator, was coiled into a secondary oven, and terminated in the source of the TOFMS. A fast, 2 µL direct injection at 300°C into a 4mm Uniliner (Restek) was used for each analysis. The primary GC oven was programmed as follows: 150°C (1 min), 60°/min to 180°, 8°/min to 320°. The secondary oven, containing the IP-Deact, was operated at a positive 20°C offset from the primary GC oven. Helium carrier gas was a constant 7.5 mL/min. Total run time was 19 min. Electron ionization at 70eV was used for TOFMS with a source temperature of 225°C, a data acquisition rate of 100 spectra/sec, and a stored mass range of 220 to 1000u.

GCxGC-TOFMS was performed on two column configurations. Both used a 10m x 0.18mm x 0.20µm Rtx-5 (Restek) as the primary column. The secondary columns were installed similarly to the IP-Deact above, and were: a 0.75m x 0.25mm x 0.15µm Stx-500 and a 2m x 0.18mm x 0.10µm Rtx-PCB, both from Restek. A variety of operational

conditions (constant flow, pressure programming, modulation times, etc.) were attempted to improve separations. Text and/or figures in the **Results and Discussion** will contain any details.

For GCxGC-ECD, BDE 209 was not considered at this time. A 1 µL, 250°C split injection of 20:1 was used for all analyses. The GCxGC column combination was a 10m x 0.18mm x 0.20µm Rtx-5 (Restek) connected via press-fit to a 1.1m x 0.10mm x 0.10µm DB-17 (J&W Scientific). The DB-17 was passed through the modulator and into a secondary oven, before being connected to the ECD. The primary oven was programmed from 80°C (0.2 min) at 4°/min to 280° (1 min), with a secondary oven offset of 20°. The modulation time was 6 sec. A constant flow of helium carrier was used at 3 mL/min. The ECD was maintained at 325°C, and used nitrogen makeup gas so that column plus makeup flow was always 150 mL/min through the detector. The ECD was operated at 50 Hz, its maximum data collection rate. The total run time was around 51 min.

Results and Discussion

Figure 1 shows a contour plot for a mix of PBDEs, including BDE 209, analyzed using the DB-5 x IP-Deact configuration. The goal was to maximize sensitivity for 209, so the modulation time, or the thermal focus/reinject time, was a relatively long 10 sec. This allowed collection of most of each congener eluting from the primary column, in the modulation zone, prior to sending it on to the TOFMS. Since only an uncoated, deactivated "column" was used in the second dimension, there is essentially no retention in that direction, resulting in the flat profile for the contour plot.

The benefit of the focus/reinject is that much of the area for a peak that was approximately 20 sec wide when analyzed using one-dimensional GC, was now concentrated in one major "slice" that was approximately 100 ms wide (**Figure 2**). By taking this approach, it was possible to establish an external standard calibration curve for BDE 209 that ranged from 5 ng/µL down to 5 pg/µL (**Figure 3**). Although the correlation coefficient for this curve was respectable, it was quite obvious that restricting the range from 5 to 500 pg/µL resulted in a better calibration at the low end for BDE 209 (**Figure 4**). It is expected that isotope dilution (or other internal standard approach) would result in even better calibration curves.

One drawback to using long modulation times to increase sensitivity for PBDEs is that first dimension separations can be compromised. This might be fatal for individual quantification of close eluting congeners in the same homolog group (e.g. tetra- BDEs 49 and 71, and penta- BDEs 100 and 119). Another possible disadvantage is that the lack of separation in the second dimension could result in matrix components interfering with BDE determinations, although their high quantification masses (e.g. 486, 564, 644, 722, and 800) usually put them in the clear of most interferences.

Demonstrating that it was possible to generate an extremely narrow peak for BDE 209 from the modulation process led to the next experiment: GCxGC to improve selectivity for PBDEs, either within homolog groups so that they could be distinguished from each other, or to help move them away from matrix components that could interfere with their identification or quantification. Unfortunately, once a second dimension column was added, even the extremely short (0.75m) and thin filmed Stx-500, the second dimension peak width for BDE 209 became extremely broad (over 1 sec wide) as seen in **Figure 5**. This has a dramatic effect on sensitivity because the acquisition rate for the TOFMS is set to define the narrowest peaks (the other BDE peaks were about 300 ms wide and the acquisition rate was set to 25 spectra/sec). Acquiring data at a faster rate than is necessary reduces the number of transients that are summed from the 5000 Hz pulse frequency to improve signal-to-noise. The acquisition rate cannot be varied during a run.

One thing that should be noted about **Figure 5** is that the BDE 209, which might appear to be eluting earlier in the second dimension than the other BDE congeners, is actually eluting much later. It displays the GCxGC phenomenon known as wrap-around, where a component has not eluted in the time set for the second dimension separation (the same as the modulation time). In this case the modulation time was shortened to 3 sec, versus the previous 10, to preserve first dimension separations when additional PBDE congeners were added to the standard mix.

Why is the peak for BDE 209 so broad? One possibility was that the reinjection from the modulation zone was inefficient. Refocusing a peak after elution from the primary column occurs with cool nitrogen gas blowing on the Stx-500 column. Reinjection (or desorption of a peak from the modulation zone) is accomplished by hot nitrogen gas, in this case 30°C hotter than the primary GC oven. The desorption efficiency of low volatility components can sometimes be enhanced by increasing the modulator temperature offset, but this offset should not exceed the maximum operating temperature of the column in the modulator. Since BDE 209 elutes close to the maximum temperature of the Stx-500 column, increasing the modulator temperature offset was not considered. Increasing the hot pulse time, the period of time used to desorb from the modulation zone, did not produce any noticeable benefit on the 209 peak width.

To minimize modulation zone influence on peak width for BDE 209, a short piece of IP-Deact tubing, previously shown to allow 100 ms peak width generation for 209, was placed in the modulator. The entrance end was press-fitted to the Rtx-5 primary column, and the exit end was press-fitted to the Stx-500 in the secondary oven. Interestingly, focusing on and reinjecting from IP-Deact had almost no effect on peak width for BDE 209; it stayed at approximately 1 sec. A 2m x 0.18mm x 0.10µm Rtx-PCB in the second dimension showed similar peak width results for BDE 209.

Once it became clear that optimization of GCxGC conditions was being dominated by the 209 issue, consideration for it was temporarily put aside to see if GCxGC would offer value for separating congeners that might coelute within homolog groups. The constant

column flow was adjusted down to 0.5 mL/min, establishing a moderate linear velocity versus the previous constant flow of 7.5 mL/min that was used to drive BDE 209 from the column prior to reaching its maximum operating temperature. A mix containing 3 mono-, 7 di-, 8 tri-, 6 tetra-, 7 penta-, 5 hexa-, and 3 hepta- BDEs was used for this part of the testing. **Figure 6** zooms in on the contour plot region for tetra- and penta- BDEs analyzed using the Rtx-5 and Stx-500 with the following conditions: primary oven programmed 150°C (1 min), 10°/min to 320° (1 min). The secondary oven was offset 20°C from the primary oven at all times and the modulation time was 3 sec. As can be seen from the figure, all of the separations within homolog groups are occurring in the first dimension, so no real separation benefit (at least among PBDEs) has occurred from using GCxGC. The other homolog groups in the standard showed the same trend. The Rtx-PCB in the second dimension produced similar results for homolog groups (i.e. no real second dimension separations), but a distinct separation between 126 and 154 was noted (**Figure 7**). Using MS, this is likely not very important, as these congeners can be distinguished. But it may have some consideration for GCxGC-ECD work.

The lack of success in moving congeners around in the second dimension with GCxGC was disappointing, but if important PBDE separations are occurring in the first dimension, and GCxGC can have some effect in separating bulk matrix components from PBDEs, then even with the column systems described here there will be some benefit.

When using a selective detector such as an ECD, it becomes more important to achieve good GCxGC results. One example is when polychlorinated biphenyls (PCBs) and PBDEs are located in the same samples. **Figures 8 and 9** illustrate the possibilities of GCxGC-ECD to determine PBDEs, even when PCBs are in the same extract. In a one-dimensional analysis of this same Aroclor:PBDE standard mix, coelutions would prevent accurate quantification of PBDEs (**Figure 10**).

Importantly, the ECD is extremely sensitive towards PBDES, and focusing offered with GCxGC enhances this sensitivity. For example, BDE 49, a tetrabromo-, gave a signal-to-noise ratio of about 150:1 for 0.5 pg. Obviously, low fg levels are achievable for environmental samples if interference-free separations are accomplished.

Conclusions

Both GCxGC-TOFMS and GCxGC-ECD offer exciting possibilities for PBDE determinations in environmental samples. Column combinations are needed that enhance the selectivity for PBDEs, to separate them from each other and from interfering matrix components, especially for ECD. The sensitivity improvement afforded by the focusing effect of modulation is important for all congeners, but particularly for BDE 209, which is difficult to analyze and usually exists at very low levels in biota samples. Further experimentation is needed to narrow peak widths for BDE 209 when using GCxGC. The possibility of using thermal focusing on uncoated deactivated tubing to lower the detection limit for BDE 209 was demonstrated.

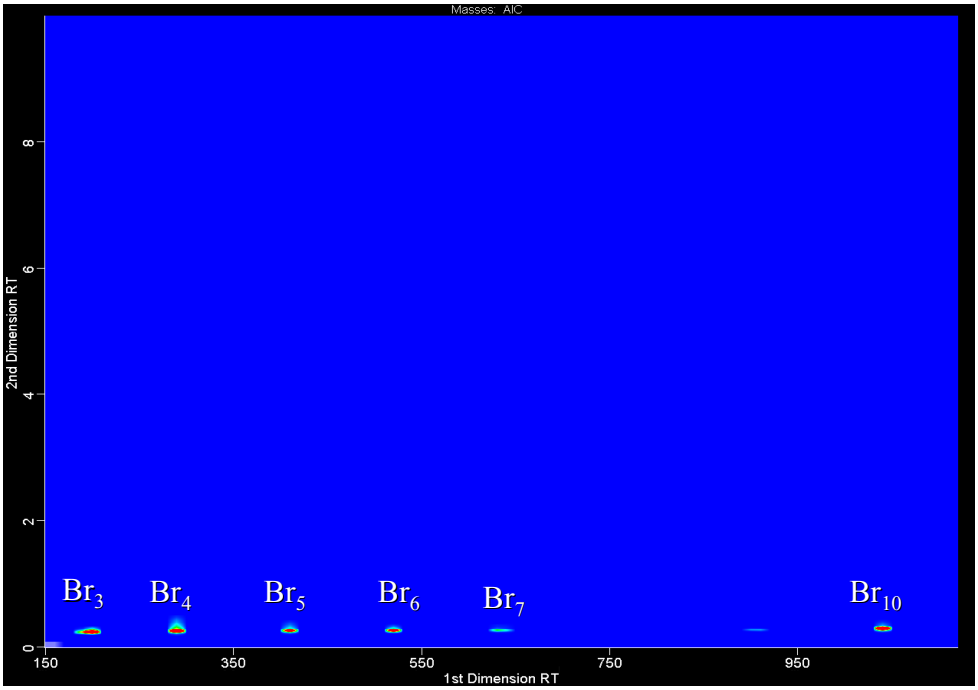

Figure 1. Contour plot of PBDE Mix containing one each of tri-, tetra-, penta-, hexa-, hepta- BDEs, and deca- BDE separated on DB-5 and then focused and reinjected to the TOFMS (from IP-Deact). A 10 sec modulation time was used to maximize collection of each congener to the modulation zone. There is essentially no retention of the BDEs by the uncoated, deactivated fused silica (second dimension).

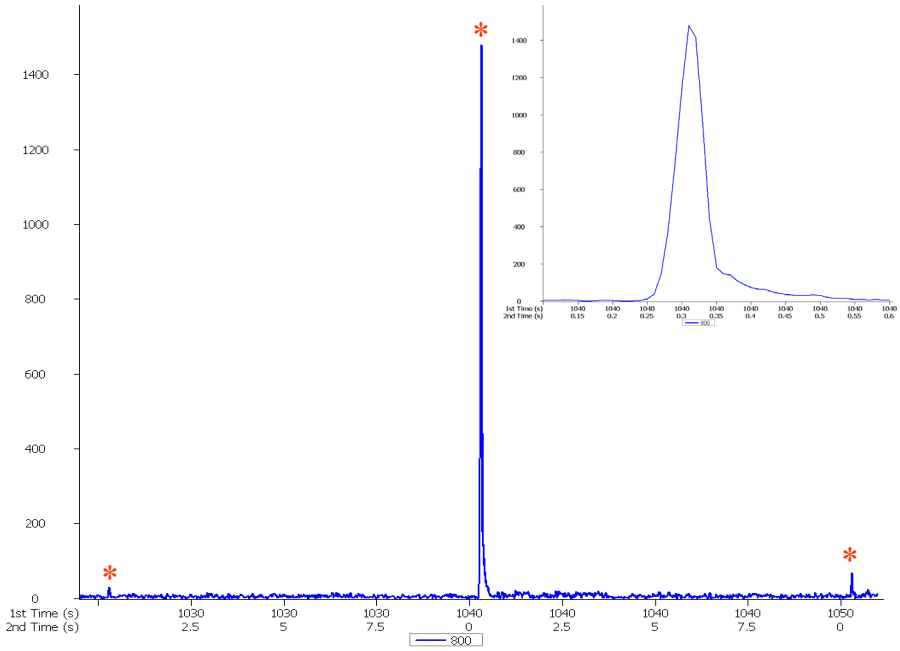

Figure 2. Modulated BDE 209 on the DB-5 x IP-Deact combination. Each of three slices is marked with an asterisk. The inset is the base slice and is approximately 100 ms wide.

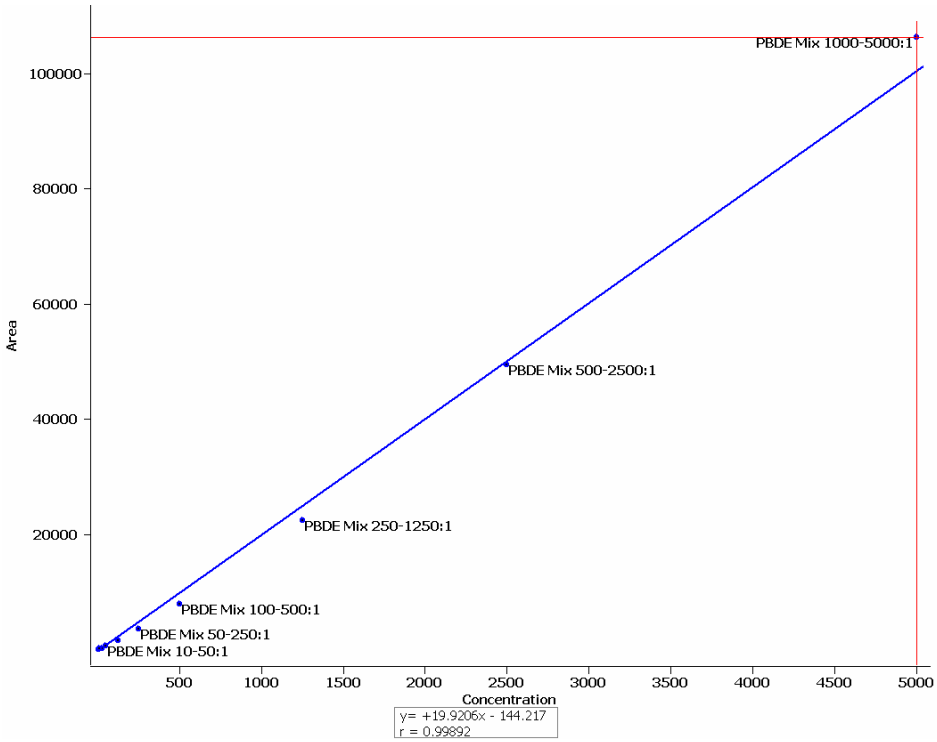

Figure 3. Calibration curve for BDE 209 ranging from 5 pg/µL to 5 ng/µL. 800 was used as the quantification mass.

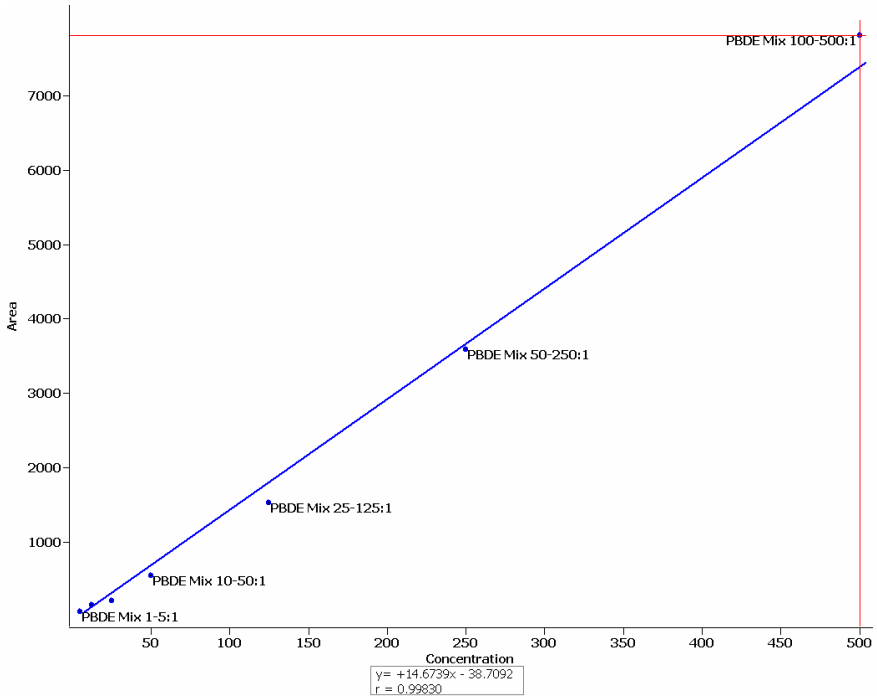

Figure 4. Calibration curve for BDE 209 ranging from 5 to 500 pg/µL. 800 was used as the quantification mass.

Error!

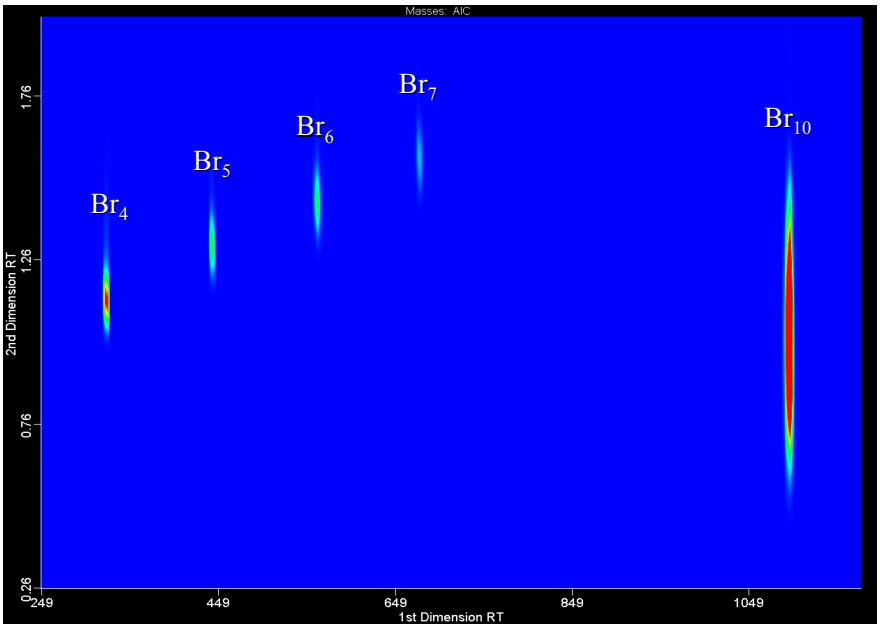

Figure 5. Zoomed contour plot showing GCxGC of PBDE mix. An Stx-500 column was used in the second dimension with a modulation time of 3 sec. The other GC conditions were the same as for the IP-Deact experiment and are in the Materials and Methods. The second dimension peak width for BDE 209 is extremely broad, over 1 sec wide, and has "wrapped around".

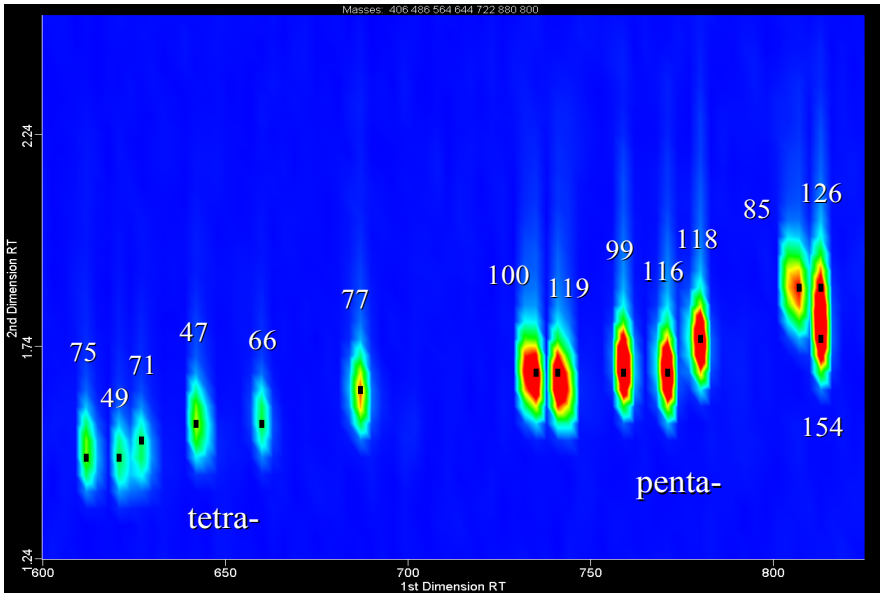

Figure 6. Zoomed contour plot of tetra- and penta- BDEs analyzed using GCxGC on Rtx-5 x Stx-500. Congener 154 is a hexa- BDE. Essentially all separations between congeners in a particular homolog group are occurring only in the first dimension.

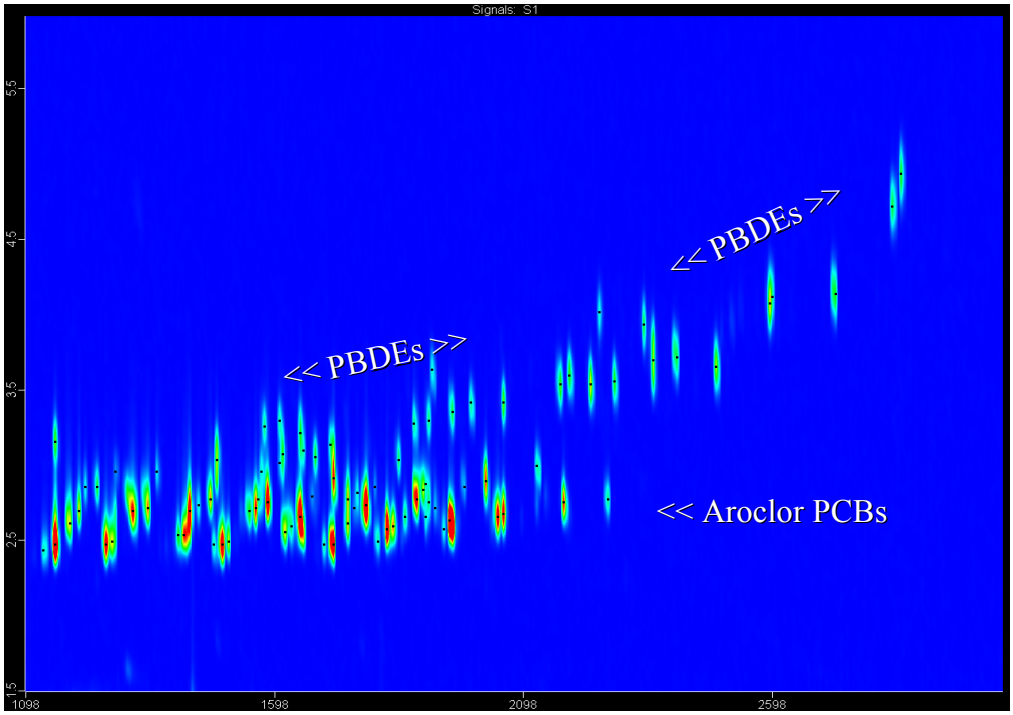

Figure 8. Contour plot showing GCxGC-ECD separation of PCBs and PBDEs.

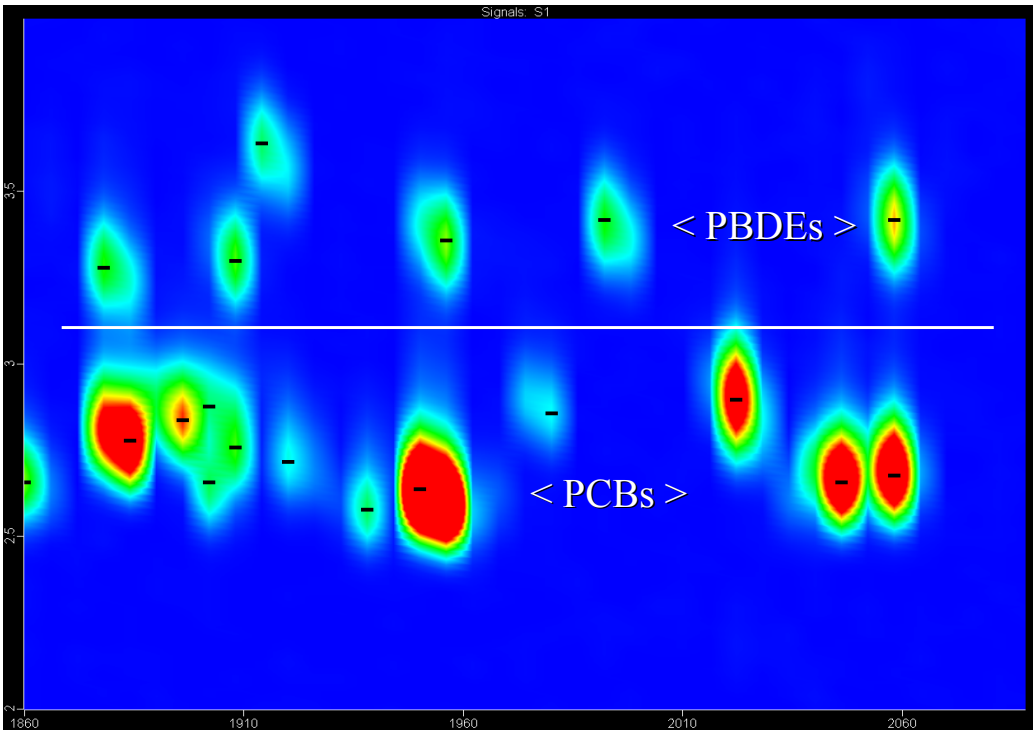

Figure 9. Zoom of previous contour plot showing distinct GCxGC-ECD separation of PCBs and PBDEs.

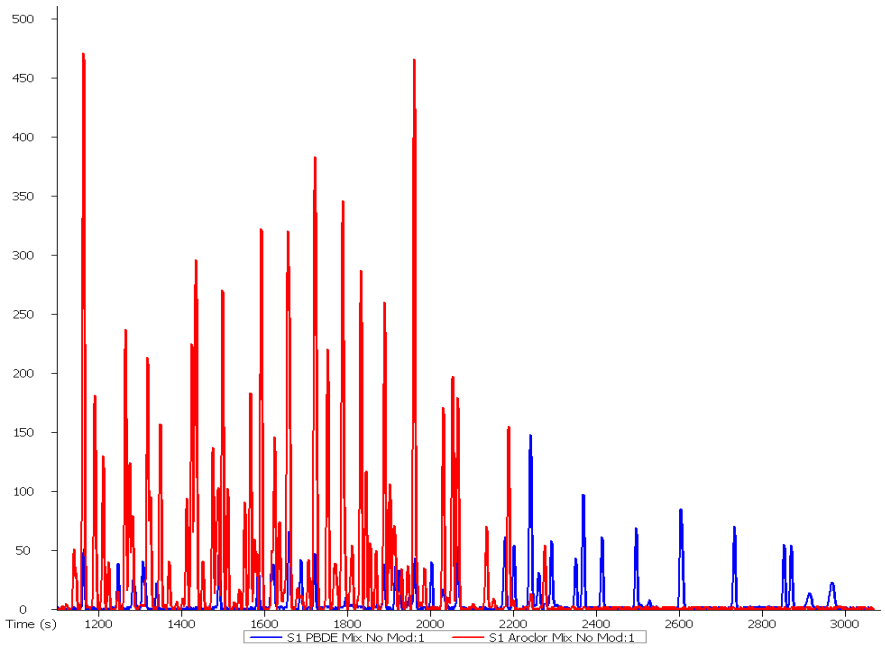

Figure 10. Overlaid one-dimensional ECD chromatograms for a PBDE standard mix (blue) and an Aroclor mix (red). Numerous coelutions occur under these GC conditions.

Acknowledgments

Ben Priest at Cambridge Isotope Laboratories kindly provided the solution that contained numerous PBDE congeners from mono- to heptabromodiphenyl ether. Mehran Alaee encouraged the presentation of this work.

References

1. Alaee, M. Fifth Annual Workshop on Brominated Flame Retardants in the Environment, Boston, Massachusetts, August 22-23, 2003.
2. Environ. Sci. Technol. Online, Science News, November 6, 2003 http://pubs.acs.org/subscribe/journals/esthag-w/2003/nov/science/kb_flame.html
3. de Boer J. and P. Cofino. Chemosphere (2002) 46, 625.
4. Kolic, T.M., K.A. MacPherson, E.J. Reiner, T. Ho, S. Kleywegt, M. Payne, and M. Alaee. Fifth Annual Workshop on Brominated Flame Retardants in the Environment, Boston, Massachusetts, August 22-23, 2003.
5. Tollbäck, P., J. Björklund, and C. Östman. Organohalogen Compounds, Volume 2, Section 1, Dioxin 2003, Boston, MA.
6. Björklund J., P. Tollbäck, and C. Östman. Organohalogen Compounds, Volume 2, Section 1, Dioxin 2003, Boston, MA.
7. Sun G., A. Yau, and T. Farias. Organohalogen Compounds, Volume 2, Section 1, Dioxin 2003, Boston, MA.
8. Shin, S.K., and T.S. Kim. Organohalogen Compounds, Volume 2, Section 1, Dioxin 2003, Boston, MA.
9. Pirard, C., E. de Pauw, and J-F. Focant. Fifth Annual Workshop on Brominated Flame Retardants in the Environment, Boston, Massachusetts, August 22-23, 2003.
10. Larrazabal, D., E. Eljarrat, D. Barcelo, and B. Fabrellas. Fifth Annual Workshop on Brominated Flame Retardants in the Environment, Boston, Massachusetts, August 22-23, 2003.
11. Cochran, J., F.L. Dorman, E. Reiner, T. Kolic, and K. MacPherson. Fifth Annual Workshop on Brominated Flame Retardants in the Environment, Boston, Massachusetts, August 22-23, 2003.