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APPLICATIONS

Fast, Accurate Analysis of Polybrominated Diphenyl Esters (PBDEs) In A Single Run, Including BDE-209

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Polybrominated Diphenyl Ethers (PBDEs) are aromatic, non-polar compounds formerly used as flame retardants. These compounds were determined to be toxic and are now included on the Stockholm Convention's list of persistent organic pollutants (POPs). Though their use has been restricted or banned in many areas, low levels of remaining PBDEs in biological, environmental, or food sources are subject to bioaccumulation and can still result in detrimental reproductive and other health effects.

This work utilizes a new method and technology that provides fast quantitation of toxic PBDE congeners with short run times and includes the quantitation of BDE-209 in a single analytical run. This improved procedure allows significant time and cost savings by eliminating the need for additional columns and instrumentation traditionally required to achieve sufficient separation of all PBDE congeners. Comparison of existing methods and the single-test method are included, highlighting improved sensitivity and shortened run times.

Introduction

PBDEs consist of 209 individual conformations called congeners, which vary in toxicity. Analytical testing of these congeners is performed by high resolution gas chromatography with high resolution mass spectrometry (HRGC/HRMS) to achieve the lowest levels of detection and highest degrees of confidence possible. Even using this advanced instrumentation, achieving accurate, well-resolved separation of all congeners proves difficult.

PBDE analysis is historically problematic for two main reasons: the sheer number of compounds and analyte stability. In addition, some congeners are thermally labile, sensitive to column activity, or both. The most notorious of these reactive congeners is BDE-209, decabromodiphenyl ether. Complete testing of BDE-209 is especially important because it can break down in the body or environment to even more toxic congeners. To obtain resolution of the all 209 congeners, many labs analyze a single list of PBDEs by two separate tests. The first uses a detailed method that resolves most congeners and traditionally employs a low polarity column of 60 m x 0.25 mm ID dimensions. However, this configuration results in nearly hour-long run times. As a result, the latest eluting congener BDE-209 frequently displays dramatically reduced peak response due to extended exposure to thermal degradation and column activity.

Labs are, therefore, often forced to analyze this compound with a second method, typically using a separate instrument and a much shorter, thinner phase column that will provide less retention. This allows a lower elution temperature and helps address thermal stability issues for BDE-209. However, thinner phase columns are more susceptible to activity, leading to peak tailing and more difficult quantitation. This work addresses the contribution of thermal stability and column activity to BDE-209 breakdown and also provides an optimized method that resolves important congeners as well as BDE-209 in one short run.

Experimental Conditions

The analytical conditions used to investigate thermal stability of BDE-209 are presented in **Table 1**. Excluding oven program (**A** and **B**), conditions were the same for both separations.

Table 1.
Analytical conditions used to collect BDE-209 thermal stability data

Column	Traditional 5 % Phenyl-Arylene Phase
Dimensions	10 meter x 0.18 mm x 0.18 µm
Injection	Split 10:1 @ 250 °C, 1 µL
Oven Program	A: 100 °C for 1 min to 300 °C @ 10 °C/min for 30 min B: 100 °C for 1 min to 250 °C @ 10 °C/min for 30 min
Carrier Gas	Helium @ 3.0 mL/min (constant flow)
Detector	Electron Capture (ECD) @ 350 °C

Analytical conditions used to separate several important PBDE congeners on GC columns from two different manufacturers are shown in **Table 2**. Instrumentation used was a HRGC/HRMS.

Table 2.
Analytical conditions for PBDE congener separations on columns from different manufacturers

Column	A: Zebron™ ZB-SemiVolatiles B: Agilent® DB-5ms Ultra Inert
Dimensions	20 meter x 0.18 mm x 0.18 µm
Injection	Splitless @ 85 °C, 5 µL
Oven Program	70 °C for 1.25 min to 240 °C @ 20 °C/min to 320 °C @ 50 °C/min for 18 min
Carrier Gas	Helium @ 0.85 mL/min (constant flow)
Detector	High Res Mass Spec (HRMS) @ 325 °C
Note	Used a PTV in Solvent Vent Mode with temperature program to 320 °C in 2 min

Results and Discussion

Breakdown of BDE-209 is often attributed solely to column activity, but this is not always the case. This is shown in **Figure 1**, where PBDEs are injected under identical conditions with the exception of the final temperature using a traditional 5 % phenyl-arylene, 10 meter x 0.18 mm ID x 0.18 µm GC column. The final temperature is elevated to 300 °C in run A but is limited to 250 °C in run B. Though BDE-209 elutes in less than 22 minutes in run A, the peak is small (with an area of only 345) and is preceded by a hump that is the degradation product. Run B has a much longer 40 minute retention time for BDE-209, allowing the analyte to react with any column activity for an extended period. Degradation actually decreases, however, resulting in a much stronger peak (with area 2731, 8 times larger), though it is more broad because of later isothermal elution. This result contradicts the theory that BDE-209 breaks down due to column activity alone and demonstrates that thermal lability also largely impacts its degradation.

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Figure 1.
Demonstration of thermal stability of congener BDE-209

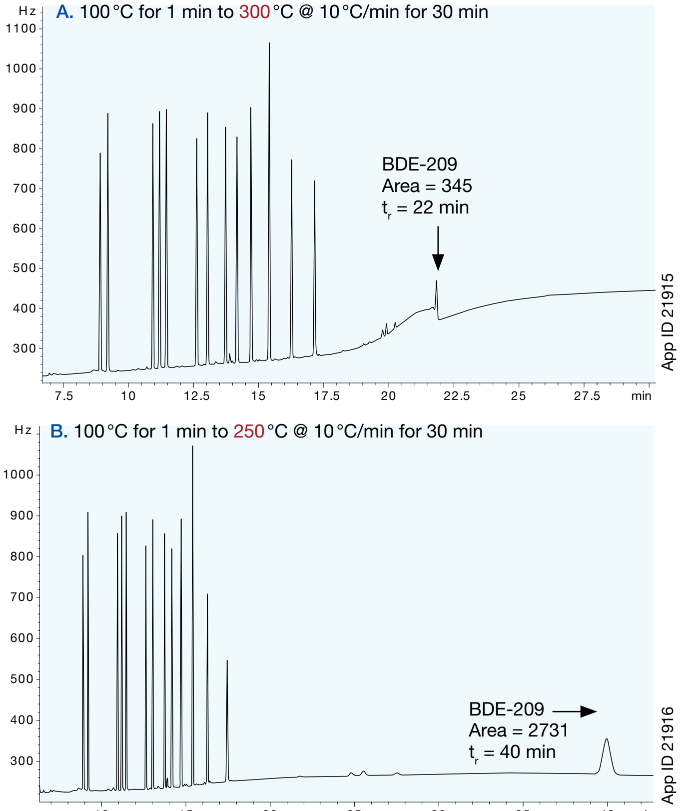
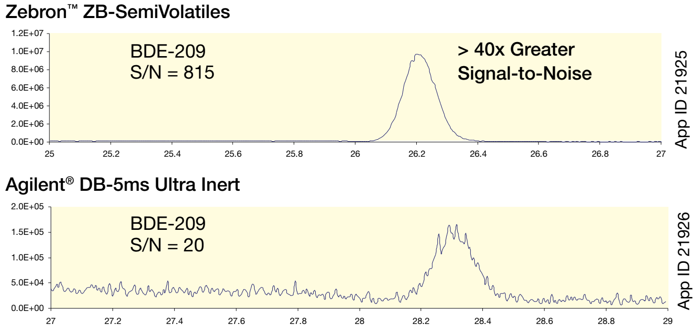
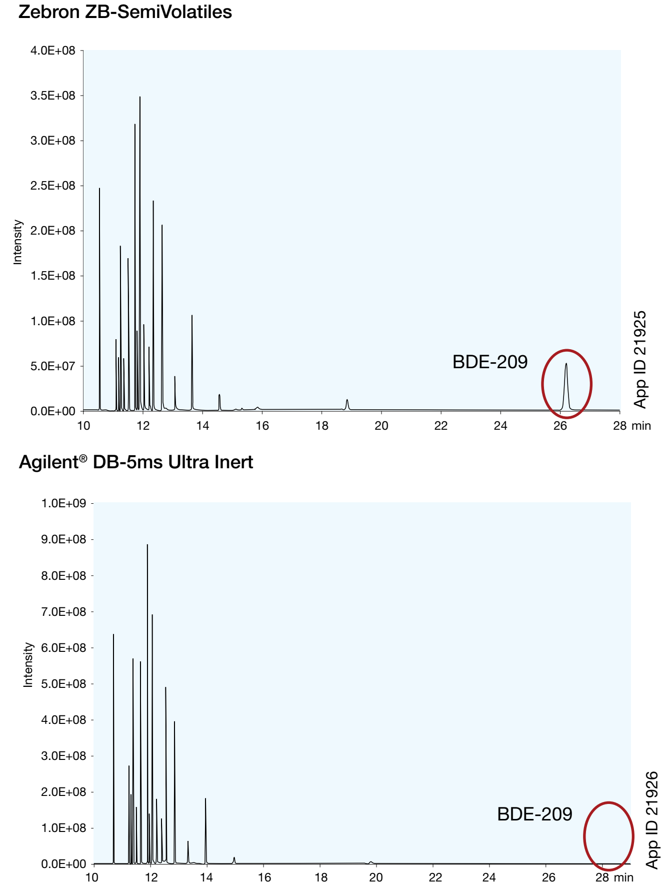


Figure 2.
Comparison of BDE-209 stability on GC columns from different manufacturers



When considered together with temperature, column activity plays a significant role in BDE-209 stability. In **Figure 2**, BDE-209 is analyzed using the same temperature program on two columns of the same dimensions, but from different manufacturers. The bottom chromatogram in the figure is an ion chromatogram for BDE-209 on a new Agilent DB-5ms Ultra Inert. The signal-to-noise (S/N) for this peak is 20. The top chromatogram shows the same analysis on a Phenomenex Zebron ZB-SemiVolatiles column, resulting in signal-to-noise of 815 for the peak – over 40 times higher than the DB-5ms Ultra Inert.

Figure 3.
PBDE responses on GC columns from two different manufacturers

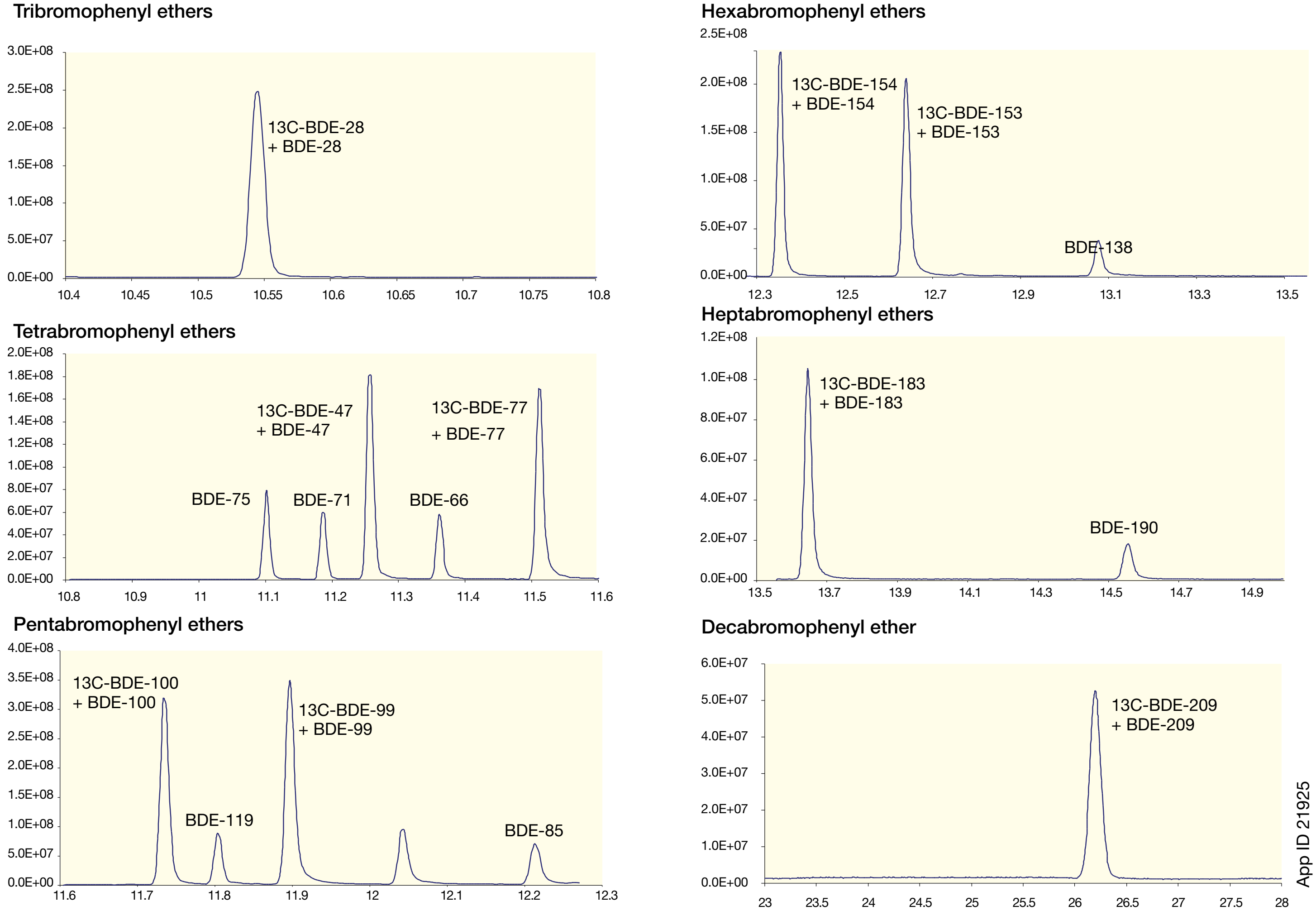


As sample, dimensions, and temperature program are the same on both runs, we can conclude that differences in column activity are affecting compound breakdown. A comparison of absolute intensities (**Figure 3**) shows that on both columns, most congeners are non-reactive and elute early enough to escape significant thermal degradation. BDE-209, however, breaks down and nearly disappears on the Agilent DB-5ms Ultra Inert, but shows a strong peak on the ZB-SemiVolatiles. Due to the inertness of ZB-SemiVolatiles BDE-209 does not degrade, demonstrating the importance of column activity in addition to temperature stability.

This improved inertness on ZB-SemiVolatiles alleviates considerable difficulties for PBDE testing by minimizing the contribution of activity to overall analyte breakdown. This allows analysts to run a single test to achieve both separation of the more toxic congeners and quantitation of BDE-209 in one run. Labs can now reallocate free HRMS systems to other projects as well as reduce the quantity of columns that need to be purchased. This has been observed in the below quote from Panu Ranatakokko, Senior Researcher at the National Institute for Health and Welfare in Finland:

"We have had great difficulties with the stability of BDE-209 with our previous GC columns, and we were forced to use a very short column (6 m) for this specific compound instead of a regular 20-30 m column. To be able to run all PBDEs in one run we decided to test Zebron ZB-SemiVolatiles.

Figure 4.
Separation of PBDE congeners on ZB-SemiVolatiles



With a narrow bore 20 m x 0.18 mm ID x 0.18 µm film ZB-SemiVolatiles column we are now able to successfully analyze our suite of PBDEs from BDE-28 to BDE-209 in a single run. Peak height of BDE-209 with this column is 10-30 times higher than with a brand new column of similar (5 % phenyl) chemistry and dimensions from another well-known manufacturer. Use of ZB-SemiVolatiles roughly halves the time required for analysis as there is no longer a need for a second injection with a shorter column.

ZB-SemiVolatiles represents a major improvement in the GC analysis of highly brominated flame retardants."

Detailed separations of other key PBDEs are provided in **Figure 4**, including tribromo-, tetrabromo-, pentabromo-, hexabromo-, heptabromo-, and decabromo- biphenyl ether congeners.

Conclusion

The lack of stability of BDE-209 has forced some labs to add a second PBDE test to analyze this compound separately. This extra testing requires additional instrumentation, extra columns, and decreases the overall productivity of the laboratory. The breakdown of BDE-209 can be attributed to a combination of both temperature stability and column activity. If a lab is able to reduce either cause, the overall response of BDE-209 can be improved.

With a narrow bore 20 m x 0.18 mm ID x 0.18 µm film Zebron ZB-SemiVolatiles GC column, labs are now able to successfully analyze a range of PBDEs from BDE-28 to BDE-209 in a single run. Use of ZB-SemiVolatiles roughly halves the time required for analysis as there is no longer a need for a second injection with a shorter column. ZB-SemiVolatiles displays superior inertness to active compounds and provides improved response when compared to other 'Ultra Inert' columns. Using the same system, sample, column dimensions, and identical conditions, signal-to-noise of BDE-209 increased over 40x when using ZB-SemiVolatiles.

Zebron ZB-SemiVolatiles represents a major improvement in the GC analysis of highly brominated flame retardants that can save labs both time and money, resulting in decreased maintenance, increased revenues, and improved productivity.

Acknowledgements

We would like to thank Panu Rantakokko from the National Institute for Health and Welfare, Finland for graciously providing data to support this work.

Ordering Infomation

Zebron™ ZB-SemiVolatiles GC Columns

ID (mm)	df (µm)	Temperature Limits (°C)	Part No.
20-Meter			
0.18	0.18	-60 to 325/350	7FD-G027-08
0.18	0.36	-60 to 325/350	7FD-G027-53
30-Meter			
0.25	0.25	-60 to 325/350	7HG-G027-11
0.25	0.50	-60 to 325/350	7HG-G027-17
60-Meter			
0.25	0.25	-60 to 325/350	7KG-G027-11

Easy Seals™ Inlet Base Seals

Part No.	Description	Unit
Standard, single groove for splitless applications, 0.8 mm dia. inlet hole		
AGO-8619	Easy Seals Inlet Base Seal, Gold Plated for Agilent GCs	2/pk
AGO-8620	Easy Seals Inlet Base Seal, Gold Plated for Agilent GCs	10/pk

PhenoRed™-400 Injector Septa

Part No.	Description	Diameter	Unit
PhenoRed-400 GuideRight™ Injector Hole Septa			
AGO-7916	PhenoRed-400, rated to 400 °C	3/8 in (9.5 mm)	50/pk
AGO-7917	PhenoRed-400, rated to 400 °C	7/16 in (9.5 mm)	50/pk

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TN16520913_W